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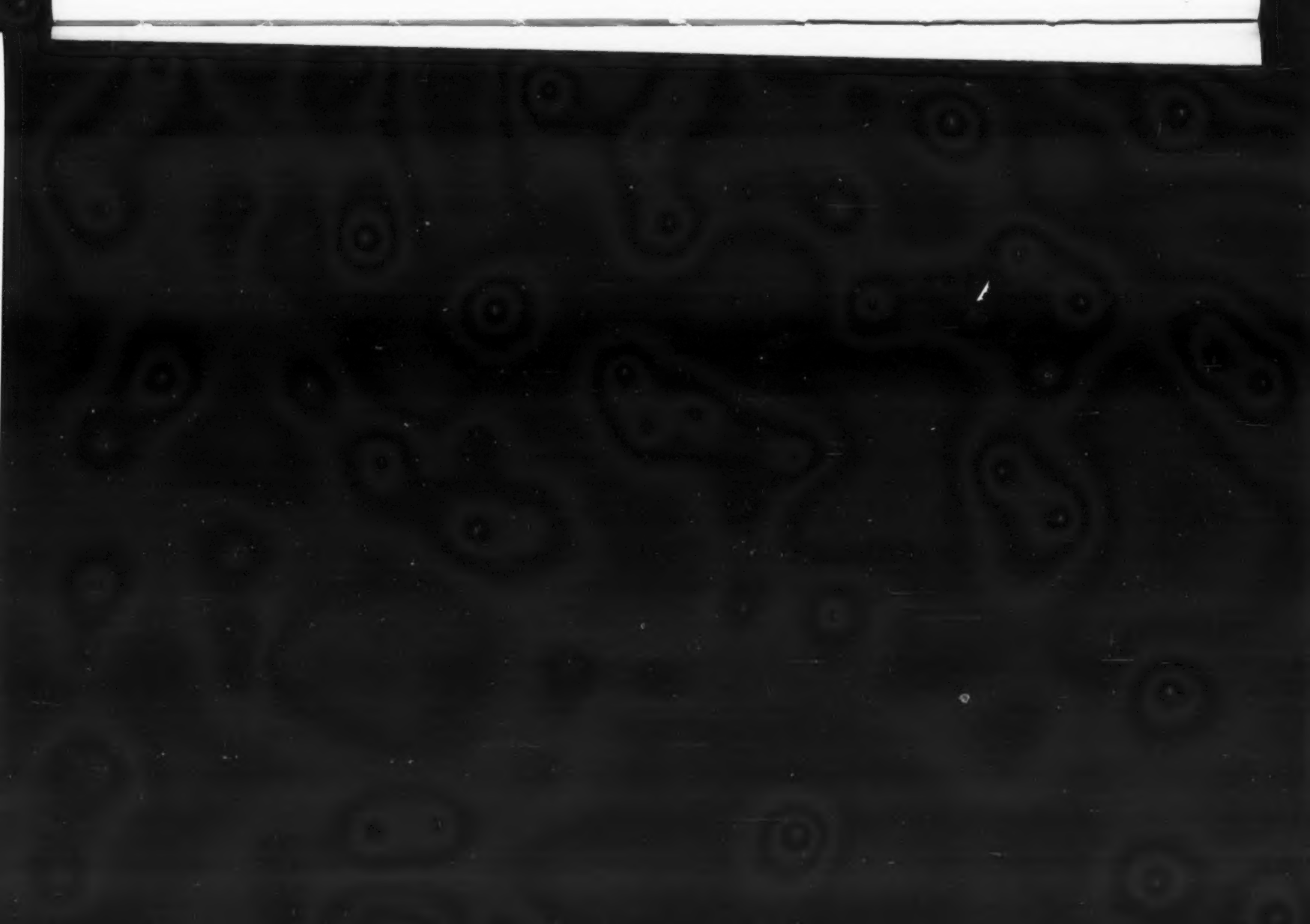

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Induction of Leukemia in Mice by Methylcholanthrene and X-Rays [1]

By D. P. McEndy, *resident in pathology*, Mary C. Boon, *research assistant*, and Jacob Furth, *associate professor of pathology, Cornell University Medical College* and *New York Hospital, New York* [2]

Numerous reports have been made on the experimental production of leukemia, and the certainty with which this disease can now be produced in mice has made it possible to study its pathogenesis. Leukemia was first produced in a mammal by Lignac (*1*), who injected benzol into the subcutaneous tissues of mice. Eight of fifty-four adult mice inoculated with 0.001 cc. of benzol in oil developed leukemia from 4 to 11 months after inoculation. One mouse had lymphoblastic leukemia; 2, aleukemic lymphomatosis; 2, mast-cell leukemia, one of which was leukemic and the other aleukemic; 2, aleukemic myelosis; and 1 eosinophilic leukemia. Büngeler (*2*) found 17 cases of leukemia in 97 animals surviving 8 months after repeated injections of indol; 4 mice had lymphatic leukemia, and 13 had myeloid leukemia. Only one instance of lymphatic leukemia was observed in a large number of control mice. These studies of Lignac and Büngeler have not been confirmed thus far.

There are several reports on the production of leukemia by carcinogenic hydrocarbons. An increase in the incidence of leukemia was noted by Burrows and Cook (*3*) following repeated injection into mice of a water-soluble succinate salt, 1,2,5,6-dibenzanthracene. Intrasplenic injection of 1,2-benzpyrene in mice was shown by workers in this laboratory (*4*) to cause an increase in the incidence of leukemia. Brues and Marble (*5*) reported that lymphomatosis increased from 2 to 50 percent in mice as a result of painting with tar. Morton and Mider (*6*, *7*) found that the cutaneous application of a benzene solution of methylcholanthrene hastened the appearance of leukemia in mice and greatly increased its incidence. Law and Lewisohn (*8*) made similar observations indicating that mice which were susceptible to the development of lymphomatosis in old age developed this disease at a much earlier age when painted with 9,10-dimethylbenzanthracene. Kirschbaum, Strong, and Gardner (*9*) found that

[1] *These investigations were supported by The International Cancer Research Foundation, The Jane Coffin Childs Memorial Fund for Medical Research, and the Lady Tata Memorial Trust.*

[2] *L. E. Stevens, W. E. Wright, and K. Scudder, members of the second-year class, participated in various phases of this study.*

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leukemia appeared earlier in a strain of mice in which spontaneous leukemia was common when methylcholanthrene was applied percutaneously. The same procedure had little effect, however, in mice with no predisposition to the disease. They concluded: "The effectiveness of methylcholanthrene in influencing the appearance of leukemia in young mice depended on the genetic susceptibility of mice to the disease" (*9, p. 289*). A similar conclusion was reached by Englebreth-Holm and Lefèvre (*10*) from experiments in which strains of mice with high and low incidence of leukemia had been injected and painted with 9,10-dimethyl-1,2-benzanthracene. Furth and Barnes (*11*), on the contrary, found that even mice of a low-leukemia stock could readily be rendered leukemic by painting with methylcholanthrene. White and Mider (*12*) found recently that feeding mice on a low cystine diet inhibits the induction of leukemia by this chemical.

In this study of the pathogenesis of induced leukemia, mice were painted with methylcholanthrene; some of the animals were killed at various intervals of painting, their tissues were examined histologically, the spleens and lymph nodes were assayed for the presence of malignant cells by inoculations into young, normal, susceptible mice, and the metabolism of samples of these organs was studied by Burk and associates (*13*). In a few experiments the possible synergistic effect of two leukogenic agents, X-ray and methylcholanthrene, was investigated.

MATERIALS AND METHODS

In the first experiment mice of the low-leukemia stock Rf were used, and in all subsequent experiments hybrids between low-leukemia stock Rf and high-leukemia stock Ak were used. The incidence of spontaneous leukemia in the Rf stock is approximately 3 percent and in the Rf/Ak hybrids 12 percent.

Beginning at the age of from 3 to 6 weeks, mice were painted with a 0.5-percent solution of methylcholanthrene in benzol twice a week. Alternate sites were used in order to minimize the development of skin tumors. In all cases litter mates were used as controls.

Tissue particles were minced in Tyrode's solution under aseptic conditions, and 0.1 cc. of the filtered cell suspension was injected intravenously into young, normal mice. Some irregularity with regard to the interval at which the transmissions were attempted was owing to the desirability of making these experiments at a time when the metabolism studies could be carried out.

The mice were irradiated 18 days after painting was begun in experiment 2, and in experiment 3 on the day preceding the first painting. In all instances the following factors were used: 140 kv., 5 milliamperes, 30 cm. T. S. D. (target skin distance), with an inherent filter of 1 mm. aluminum. Under these conditions the output of the machine was 100 r in 0.7 minutes. All irradiated mice received 300 r.

RESULTS

Four experiments were performed. The results are summarized in tables 1–5.

Experiment 1

Thirty-seven mice of stock Rf were painted. Approximately three mice were killed weekly after from 7 to 23 weeks of painting with methylcholanthrene. The results are given in table 1. Transmission of leukemia from mice which developed the disease was successful in all four attempts. The first instance of leukemia was recognized on gross and micro-

TABLE 1.—*Result of painting mice with methylcholanthrene*[1]

Stock of mice	Findings after beginning of painting (in weeks)																
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Rf	– – – – – – – – –	– – – – – – – –	– – – – – – – –	– – – – – – – –	– – – – – – – –	– – – – – + + +	– – – – – – – –	– – – – – – – – +	– – – – – – – –	+ + + + + + +	+ + +			– – –	+ + +	– –	+ + – – –

[1] Each group of 2 or 3 plus or minus signs represents a mouse. The first symbol indicates the results of gross examination; the second, the microscopic examination; the third, the transmission experiments; + sign indicates leukemia; – sign, absence of leukemia or questionable leukemia. E. g., +++ indicates that gross and microscopic examinations and the transmission experiment indicated leukemia; – + indicates that the gross examination was negative, the microscopic examination positive for leukemia, and transmission was not attempted. Each of the three observations was made without cognizance of the other two.

scopic examination, and transmission was successful in all six injected mice. A transfer was not attempted from the second case of leukemia. Three mice examined after 16 weeks of painting had leukemia, and all were recognized on gross examination. Attempted transmission of one case was successful, all six of the mice injected developing the disease. One mouse had leukemia after 17 weeks of painting, and the disease was transmitted to all six mice injected.

All inoculation experiments were successful from the 4 mice in which leukemia was recognized on gross examination. Transmission experiments were made after from 7 to 23 weeks of painting from 19 different mice in which leukemia was not found on gross or microscopic examination, each into approximately 6 normal mice. These inoculations were uniformly unsuccessful.

Fifteen siblings of the painted mice left untreated as controls are not listed in table 1. They were killed from 2 to 12 months after painting of their siblings had begun, and none of these mice had leukemia. Eleven mice which were examined within 16 weeks after the beginning of the experiment showed normal organs, both on gross and on microscopic examination. Three killed at 6 months of age showed moderate lymphoid hyperplasia. One killed at 1 year had lymphoid hyperplasia with questionable lymphomatosis and a lung tumor. This was the only mouse of the control group which had a lung tumor.

Papillomas began to appear in mice after 12 weeks of painting and carcinomas after 13 weeks; all mice killed after 12 weeks had either cutaneous papillomas or carcinomas or both. Lung tumors were found in all but three mice of this series; these three were killed 7, 8, and 9 weeks after painting, respectively.

Experiment 1 shows a perfect correlation between transmissibility and anatomical evidence of leukemia. All instances of anatomically evident leukemia proved transplantable to all mice injected. Inoculations with cell suspension from the spleen and lymph node of painted mice which showed no anatomical evidence of leukemia were uniformly negative. Since earlier studies have shown that a small number of cells are sufficient to transplant leukemia of mice (*14*), it can be assumed that the malignant transformation in any given animal is relatively sudden, even though in time of appearance somewhat variable from animal to animal, and that the course of the illness is relatively rapid.

Most painted mice which did not have leukemia showed hyperplastic changes in lymph nodes and spleen, with large lymphoid follicles and many mitotic figures in the germinal centers. There was nonmalignant extramedullary myelopoiesis in the spleens of the painted animals in excess of that seen in the controls. No significant changes were seen in the liver or bone marrow of these mice. Transmission experiments were carried out with cell suspensions from the spleen and the lymph nodes of several of these mice, but not a single case of leukemia was produced.

Experiment 2

Experiment 2 was similar to experiment 1. Rf/Ak hybrids were used. Approximately one-half of the mice were irradiated 2 weeks after painting had begun. The experiment was complicated by the loss of a large number of unirradiated mice as the result of fighting. Table 2 shows that the painting was highly effective in both irradiated mice and control mice. The unirradiated mice which lived 11 weeks or longer after the first painting had leukemia. In the irradiated group, leukemia appeared

TABLE 2.—*Result of painting X-rayed and normal mice with methylcholanthrene* [1]

Group	Treatment	Findings after beginning of painting (in weeks)															
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A	Painted	− −[2]					++		++ ++					++			
B	Painted and irradiated.			++ −	++ − ++			++ ++	++ ++	++	−+ ++ ++	++ ++ ++				− −	− −

[1] For meaning of symbols, see footnote to table 1.
[2] 12 mice.

after 8 weeks of painting. From this time on, all mice which died or were killed had leukemia during a period up to 17 weeks. It is remarkable that the irradiated mice which died after 20 and 21 weeks of painting gave no evidence of leukemia.

Transmission was attempted from only one irradiated mouse killed 9 weeks after painting. Grossly the mouse appeared healthy, but microscopic examination showed that it had lymphatic leukemia. Six of seven mice injected with mixed tissues of lymph node and spleen of this mouse died of leukemia.

EXPERIMENT 3

In experiment 3, 108 Rf/Ak mice were used, of which 25 were painted and irradiated, 24 painted only, 19 irradiated only, and 40 were left untreated. Table 3 gives the results on the first 2 groups only. Most of the mice of these 2 groups which died or were killed from 8 to 20 weeks after painting was started developed leukemia. In 3 instances the disease was recognized on microscopic examination only. One painted and irradiated animal that died at 8 months and another that died at 9 months showed no leukemia on gross and microscopic examinations.

Two attempts at transmission were made, both by intravenous injections of combined lymph node and spleen suspensions from mice that had been irradiated and painted for 5 and 6 weeks, respectively. The gross and microscopic examination of the organs of the donor mice gave no evidence of leukemia, and the injections into a total of 11 mice were negative.

Twenty unirradiated and unpainted mice (not listed in table 3), examined during a period of from 5 weeks to 8 months after painting of their siblings was started, were negative for leukemia on gross and microscopic examinations. Twenty-two additional control mice are still living and are negative at over 12 months.

Four irradiated control mice died with leukemia at 5, 7, 8, and 10 months, respectively; 1 mouse was negative after 6 weeks; the remaining 14 animals are alive and seemingly healthy at 12 months.

In this experiment pre-irradiation may have hastened the onset of leukemia induced by methylcholanthrene. Leukemia appeared first in the irradiated group 10 weeks after painting had begun, and 2 weeks later in unirradiated mice. The two mice from which transfers were attempted at 5 and 6 weeks after painting had begun did not contain malignant blood cells, at least not in numbers sufficient to be demonstrable by inoculation tests.

Four irradiated unpainted controls developed the disease, although at a much later time than the painted mice. Previous experiments indicated that X-rays are leukemogenic (*15*, *16*).

TABLE 3.—*Effect of painting mice with methylcholanthrene* [1]

Group	Treatment	Findings after beginning of painting (in weeks)																		
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A	Painted			− − −

Experiment 4

Twenty-five unirradiated Rf/Ak mice were painted with methylcholanthrene. Twenty-four siblings were kept as controls. The results are given in table 4. Four mice killed for metabolism studies after 7 weeks of painting were negative for leukemia on gross and microscopic examinations. After 10 weeks of painting 2 mice died of leukemia which was evident on both gross and microscopic examinations. During the period of from 11 to 15 weeks, 15 of the 19 painted mice which died or were killed had leukemia. Of these 8 were positive for leukemia on gross and on microscopic examination, 3 on microscopic examination only. Twelve unpainted control mice died or were killed from the fourth week to the tenth month after painting of their siblings had been started; none had leukemia. Twelve are still living.

Transmission experiments were made at 12 to 14 weeks after painting. Those from 2 cases, in which both the gross and the microscopic examination indicated leukemia, were successful in all 13 mice that were injected, while that from a mouse which showed no leukemic change gave no leukemia in the 6 injected mice.

Earliest Leukemic Change Recognized on Microscopic Examination

Three additional transmission experiments were carried out in experiment 4 using tissues from animals which were considered negative on gross examination and at first negative or questionably leukemic on microscopic examination. The results of inoculations were not consistently positive, but many mice injected with tissues from these three mice developed leukemia. A subsequent review of the microscopic sections revealed the presence of groups of malignant cells in the lymph nodes and spleens of these painted mice. Since these cases represent the earliest malignant change thus far proved by inoculation experiment, they will be described in detail.

Table 4.—*Result of painting unirradiated mice with methylcholanthrene* [1]

Stock	Findings after beginning of painting (in weeks)							
	7	8	9	10	11	12	13	14
Rf/Ak	− − − − − − − −			+ + + +	− +	− − + + + + +	− + + + + + − − + + + + + + + + − − − − − − −	− − + + + + + + + − − +

[1] For meaning of symbols see footnote to table 1.

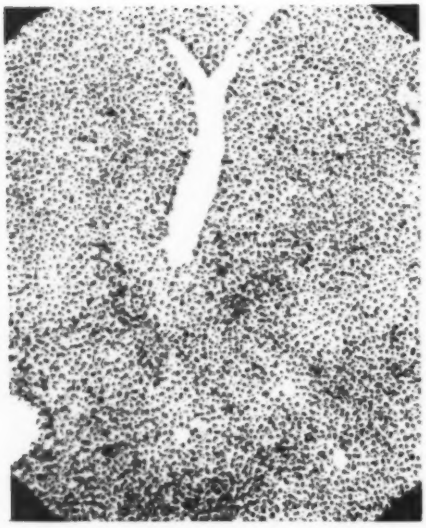

FIGURE 1.—Liver from mouse Mb 104, showing absence of leukemia. (Transmission experiments with the spleen of this mouse were positive for leukemia.) The livers from the other two mice with incipient leukemia were similar to this. Haematoxylin and eosin. × 200.

Case 1 (Mb 104).—Mouse Mb 104 was killed after 12 weeks of painting. At autopsy the lymph nodes were not enlarged, the cervical, axillary, and inguinal lymph nodes measuring approximately 3 mm. in greatest diameter. The spleen was slightly enlarged and measured 17 x 6 mm. The liver showed no change. On routine microscopic examination the liver (fig. 1), lymph nodes (fig. 2), and bone marrow were considered negative for leukemia, although the spleen was questionable. When the sections were reexamined, it was seen that the splenic pulp contained many large cells with irregularly lobulated or indented nuclei (figs. 3, 4). Mitotic figures were numerous among the atypical cells.

At no place was the capsule infiltrated by these cells, and the general architectural outlines remained, although the follicular outlines were indistinct. Erythrogenic cells were present in numerous small, scattered foci. Definite myeloid cells were almost absent. Only an occasional megakaryocyte was present. The lesions may be regarded as indicative of leukemia of the atypical cell type. The lymph node was approximately normal in size, and its structure was well-preserved. The lymphoid follicles were of normal size. Germ centers were present with few mitotic figures. In and about the reticular framework cells of the organ, an occasional atypical mononuclear cell was found similar to those seen in the spleen. There were several mitotic figures. The reticulo-endothelial framework cells of the organ were swollen; their nuclei were

FIGURE 2.—Lymph node from mouse Mb 104, showing a large lymphoid follicle with a large germ center, open marginal sinus, and hyperplastic but seemingly not leukemic medulla. Haematoxylin and eosin. × 150.

large and pale-staining; the cytoplasm was very pale staining, indistinct, and "foamy." The relation of the mononuclear leukemic cells to those of the reticular framework is uncertain.

Four mice were injected with spleen, and three with lymph node suspensions. Two of the mice injected with cells from the spleen were healthy when killed 105 days after injection. One that died 99 days after injection was leukemic on gross examination, and the fourth mouse, which died on the same day, on microscopic examination suggested early leukemia. All of the three animals injected with cells from minced lymph node were normal when killed 105 days later.

Case 2 (Mb 102).—Mouse Mb 102 was killed 14 weeks after painting had begun. At autopsy the cervical, axillary, inguinal, and retroperitoneal lymph nodes were slightly enlarged and averaged 3 mm. in diameter. The spleen measured 15 x 4 mm. and was also slightly enlarged. In general, the architecture of the spleen was well-preserved. The lymphoid follicles were large and well-defined. The pulp contained numerous scattered erythrogenic foci; but among these were scattered focal accumulations of large lymphoid cells with several atypical forms that were indistinguishable from the atypical cells of malignant leukemias. Only occasional small myelogenic foci were encountered. Throughout the pulp were large yellow pigment-laden monocytes. The structure of the lymph node was well-preserved.

FIGURE 3.—Spleen from mouse Mb 104. The malpighian follicle (left lower quadrant) is reduced in size. There are scattered focal accumulations of erythroblasts conspicuous about the upper margin of the figure, but the bulk of the field is occupied by large mononuclear cells. Haematoxylin and eosin. × 150.

In most parts the reticulo-endothelial cells of the framework of the node were large and swollen, as described previously. The lymph sinuses were open. The follicles were large and were composed of small lymphocytes, with perhaps a slightly greater number of large lymphocytes than normal. Scattered throughout the node

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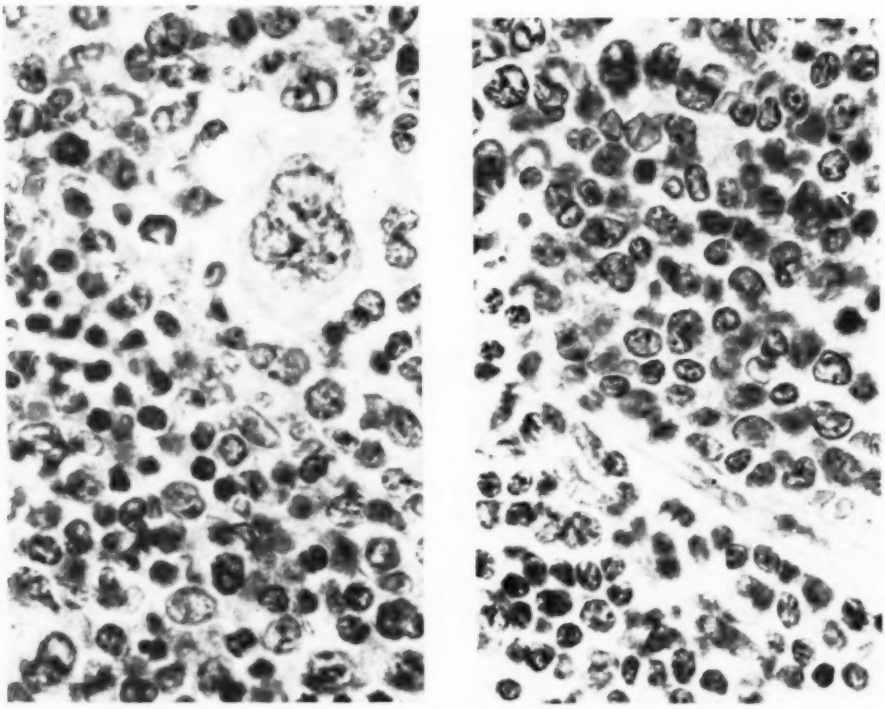

FIGURE 4, *A*, and *B*.—Pulp of the spleen of mouse Mb 104 (shown with low power in figure 3). There are numerous erythroblasts in the left middle field of figure 4, *A*, and a megakaryocyte in the right upper field. Characteristic atypical, presumably malignant cells are present in both fields. Haematoxylin and eosin. × 900.

were several mitotic figures among larger lymphoid cells, but there was no great accumulation of these cells. The liver was entirely negative on microscopic examination. The bone marrow showed only granulocytic hyperplasia (fig. 5).

Four young normal mice were injected with cell suspension from the spleen, and all died of leukemia from 34 to 39 days after injection. Of three mice injected with lymph node suspension, two died of leukemia 78 and 90 days after injection, respectively.

Case 3 (*Mb 86*).—Mouse Mb 86, killed 14 weeks after painting was begun, was negative on gross and questionably leukemic on microscopic examination. The spleen measured 15 x 5 mm. in the two greatest diameters. The structure of the organ was retained, although the follicles were small. There were numerous large areas of erythropoietic cells with many megakaryocytes in the pulp (fig. 6, *A*) and between them were large round cells (fig. 6, *B*) of the approximate size and shape of myelocytes. Their nuclei were

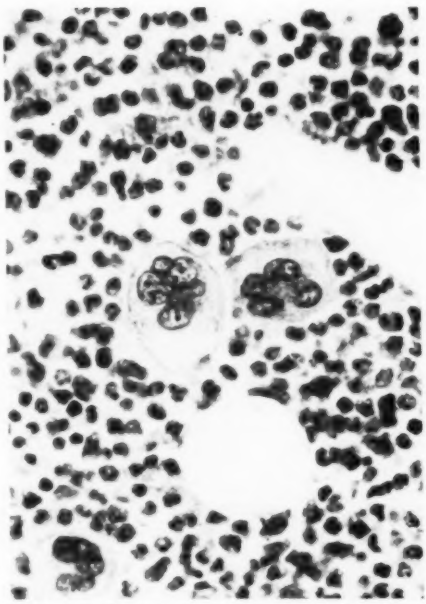

FIGURE 5.—Bone marrow from mouse Mb 102 showing hyperplasia of granulocytes with maturation and three megakaryocytes. Haematoxylin and eosin. × 600.

often indented and convoluted like those found in atypical leukemia (*17*). There were numerous mitotic figures among these cells. The liver was moderately enlarged but was normal microscopically. Most of the cells in the bone marrow were of the usual normal myeloid type. All but the mesenteric nodes were slightly enlarged, averaging 3 to 4 mm. in diameter. On microscopic examination of a cervical lymph node the sinuses were indistinct or absent, and lymphocytes of the normal type occupied the bulk of the node. Germ-centerlike areas containing large round cells with numerous mitotic figures (approximately 10 at ×90) were conspicuous. The impression is that they had partially replaced the lymph follicles. Many of the lymphoid cells were unusually large and varied more in shape and size than normally. Throughout the remaining part of the node were numerous large lymphoid cells with ameboid outline; there were numerous mitotic figures among them. Many of the large lymphoid cells described might be malignant, but on a histologic basis leukemia could not be diagnosed with certainty.

Six mice were injected, three with a suspension of cells from the spleen, three with that from lymph nodes. Of the former, two died of leukemia, 49 and 65 days after injection, respectively. Two mice injected with a cell suspension from lymph nodes died 14 and 96 days after injection, respectively, and were negative for leukemia in the gross. The third died after 51 days with generalized enlargement of lymph nodes and the microscopic changes of early leukemia.

Having learned about the earliest changes in induced leukemia, we reexamined the slides classified in our tables as negative. A few of those of mice painted for longer than 8 weeks showed changes similar to those seen in the spleens of these three mice; but, since no transmission experiments were made, the original diagnoses were left unchanged.

The essential difference between the earliest leukemic change and advanced nonmalignant myelopoiesis in the spleen is indicated by a comparison of our figures with those of figure 20 of Barnes and Sisman (*18*). In nonleukemic extramedullary myelopoiesis the large cells between the erythroblasts are myeloid cells in various stages of maturity (fig. 20 of Barnes and Sisman), whereas in the early induced leukemias the cells are large mononuclear cells, often with bizarre-shaped nuclei, as shown in our figures 4, and 6, *B*.

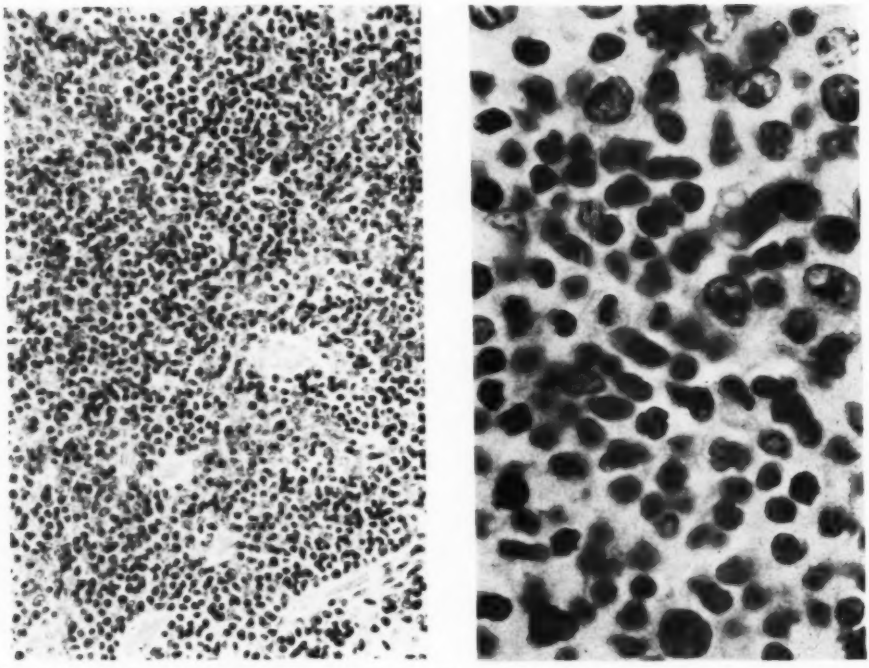

FIGURE 6.—Spleen of mouse Mb 86. *A*, Approximately one-half of the cells are erythroblasts; between these are large, pale, mononuclear cells. Haematoxylin and eosin. ×200; *B*, Atypical, presumably malignant cells. Haematoxylin and eosin. × 900.

EXPERIMENTS 1–4 SUMMARIZED

In summarizing our experiments (table 5), it is evident that the dose of methylcholanthrene used was too large for the demonstration of the synergistic action of X-rays. Methylcholanthrene alone produced leukemia in 58 percent of the painted mice that died or were killed after the seventh month of painting, while 79 percent of the painted and irradiated mice developed leukemia in the corresponding period. Three cases of leukemias developed at 8 or 9 weeks of painting in animals that were irradiated 1 day before painting had begun and none in the six unirradiated and painted mice. Should these experiments be repeated, it would be desirable to use a smaller dose of methylcholanthrene. The data suggest that the two carcinogens are synergistic, but it would be important to determine whether there is marked summation of threshold doses of these agents.

TABLE 5.—*Summary of data from tables 1–4*

Mice	Leukemia	Cases after beginning of painting (in weeks)												Mice developing leukemia
		6/7	8	9	10	11	12	13	14	15	16	17	18 and over	Total [1]	
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*
Painted	−	20	3	3	3	3	2	7	4	3	0	0	3	31	
	+	0	0	0	2	2	8	9	8	4	4	3	3	43	58
Total														74	
Painted and irradiated.	−	1	2	1	0	1	0	1	0	0	0	0	3	8	
	+	0	1	2	1	1	4	4	1	6	5	0	4	29	78
Total														37	

[1] Omitting the mice killed at 6 or 7 weeks.

PRELIMINARY HEMATOLOGICAL OBSERVATIONS

A systematic study of changes in the blood pictures during the early phases of leukemogenesis was beyond the scope of this work. Previous experiments showed that even transmissible leukemia with a high leukocyte count has a latent period during which tissues are infiltrated with malignant cells, but blood changes are absent. Hence a study of the preleukemic and early leukemic changes, which was the purpose of this investigation, did not actually require a study of the alterations in the circulating blood. Moreover, the blood involvement in leukemias occurring in our stocks of mice is variable, and the majority of spontaneous mouse leukemias are subleukemic or aleukemic in spite of the presence of greatly enlarged lymph nodes and spleens.

Nevertheless, it seemed desirable to find out the frequency, character, and time of appearance of blood changes in relation to organ changes, and to extend the assay for malignant cells to blood as well as bone marrow or other organs thus far not assayed that might be the primary site of a malignant change. In the course of the present study numerous blood smears were taken. An analysis of these smears is given in table 6.

TABLE 6.—*Analysis of blood smears from mice painted with methylcholanthrene*

Mouse No.	Treatment of mouse				Differential count											Interpretation of blood smear
	Period after painting	Period painted and fate [1]	Diagnosis [2]	Type of leukemia	Lymphocytes			Monocytes or lymphocytes	Atypical cells [3]	Polynuclears		Monocytes	Smudged cells per 100 white blood corpuscles	Polychromatophilia [4]	Erythroblasts per 100 white blood corpuscles	Estimated white cell count [5]	
					Small	Medium	Large			Mature	Immature						
	Weeks	*Weeks*			*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
Normal [6]					49	6	0	0	0	40	0	5	21	0	0	n	Normal.
Mb 57	7	7 K	− − −		49	7	0	0	0	42	0	2	22	0	0	n	Do.
Mb 59	7	7 K	− − −		52	7	1	0	0	37	0	3	16	±	0	n	Do.
Mb 65	7	7 K	− − −		34	6	0	0	0	57	0	3	34	0	0	s. i	Slight polynucleosis.
Mb 77	7	7 K	− − −		44	3	0	1	0	44	1	7	17	0	0	n	Normal.
Mb 53	7	11 D	− +	Atypical	57	7	0	1	0	32	0	3	60	0	0	n	Do.
Mb 5	8	10 K	+ +	Monocytic and atypical.	78	6	0	1	0	12	0	3	120	±	0	n. or s. i	Slight lymphocytosis.
Ma 785	9	11 K	+ +	Lymphoid	68	26	2	0	0	3	0	1	98	±	0	g. i	Lymphoid leukemia.
Ma 796	10	12 K	+ +	Atypical	55	16	1	2	1	20	1	4	74	+	0	s. i	Subleukemic lymphomatosis.
Ma 781	10	12 D	+ +	Lymphoid or atypical.	47	15	2	1	2	27	3	3	53	+	0	s. i	Subleukemic lymphomatosis with many slightly atypical cells.
Ma 805	10	13 D	+ +	Lymphoid	43	6	0	1	0	45	2	3	69	+ +	0	s. d	Anemia, leukopenia.
Ma 810	10	12 K	+ +	do	27	4	0	3	0	61	1	4	62	0	0	m. i	Subleukemic lymphomatosis.
Mb 55	11	13 K	+ +	do	60	23	3	0	3	9	1	1	40	±	0	g. i	Leukemic lymphomatosis.
Mb 94	11	13 D	+ +	Monocytic	14	11	2	3	1	14	1	54	44	+	0	s. i	Monocytic leukemia (?).
Ma 806	11	15 D	+ +	Lymphoid	57	16	2	0	2	22	0	1	66	0	0	s. i	Subleukemic lymphomatosis.
Mb 49	12	14 K	+ +	Myeloid or atypical or mixed.	35	52	4	0	9	0	0	0	26	0	0	m. i	Leukemic lymphomatosis with many slightly atypical cells.
Mb 75	12	12 K	+ +	Lymphoid	40	47	3	0	1	4	0	5	55	±	0	g. i	Leukemic lymphomatosis.
Ma 767	13	13 K	+ +	do	56	39	2	0	1	1	0	1	27	0	0	g. i	Do.
Ma 760	13	15 D	+ +	Atypical	56	22	0	1	0	5	3	13	39	0	0	s. d	Subleukemic lymphomatosis.
Ma 752	14	16 D	+ +	Lymphoid	47	4	0	1	1	33	4	10	96	0	0	n. or s. d	Normal.
Ma 768	15	15 K	+ +	Myeloid (?)	58	30	8	0	1	6	2	0	44	+ +	14	g. i	Leukemic lymphomatosis with marrow involvement.
Rff 245	16	20 K	− − −		27	4	0	0	0	68	0	1	20	±	0	n	Normal.
Rff 262	17	17 K	+ + +	Lymphoid	70	22	3	0	0	4	0	1	40	±	0	s. i	Subleukemic lymphomatosis.
Rff 246	17	21 K	+ + +	do	28	5	0	0	0	66	1	0	86	+ +	1	n	Anemia.
Rff 244	17	23 D	+ +	do	33	15	0	0	0	47	5	0	11	0	0	n	± for lymphomatosis.
Rff 247	17	23 K	− − −		42	14	1	0	0	42	1	0	28	0	0	n. or s. d	Do.
Rff 243	17	22 D	− −		30	3	0	1	0	65	1	0	32	+	0	s. i	Normal.

Rff 246	19	21	K	+ + +	Lymphoid	12	13	2	0	2	68	3	0	139	+ +	0	s. i	Anemia; ± for lymphomatosis.
Rff 245	20	20	K	− − −		30	19	1	0	0	43	7	0	14	+ +	0	s. i	Do.
Rff 246	20	21	K	+ + +	Lymphoid	27	41	6	0	1	23	1	1	46	+ +	0	m. i	Leukemic lymphomatosis.
Rff 246	21	21	K	+ + +	do	16	32	0	0	0	48	3	1	121	+ +	1	m. i	Do.
Rff 243	21	22	D	− −		19	12	0	0	1	63	4	1	14	±	0	n	± for leukemia.
Rff 244	21	23	D	+ +	Lymphoid	14	17	3	2	2	56	5	1	61	±	0	s. i	Do.
Rff 247	21	23	K	− − −		18	9	0	0	0	69	3	1	32	+	0	s. i	Do.
Rff 247	23	23	K	− − −		14	14	2	0	0	63	7	0	30	+	0	n. or s. i	Do.

[1] K, killed; D, died.
[2] For interpretation of the + and − signs, see footnote to table 1.
[3] Fully described in the text.
[4] ±, occasional; +, few; + +, numerous polychromatophilic erythrocytes.
[5] n., normal; s. i., 20,000 to 50,000 white blood corpuscles per cubic millimeter; m. i., 50,000 to 100,000 white blood corpuscles per cubic millimeter; g. i., more than 100,000 white blood corpuscles per cubic millimeter; s. d., fewer than 5,000 white blood corpuscles per cubic millimeter.
[6] Average of counts from 5 normal mice 2½ to 7½ months old.
[7] 1 in mitosis.

Contrary to our expectations, the majority of induced leukemias were accompanied by definite leukemic blood changes preceding the death of the animals by a few weeks. This leukemia was characterized by the appearance of numerous basophilic lymphocytes in the blood, most of which were of medium size, a few were large. Since cells of the latter type are practically absent in normal blood, their presence is of particular diagnostic value. The total leukocyte count can be estimated to exceed 50,000 in most cases. Anemia as suggested by polychromatophilia is not an early change (in accordance with histologic findings in the bone marrow), and in only three animals were erythroblasts found in the blood smears. Unquestionably atypical cells appeared in the blood of a few mice examined, but the same smears showed also an increase of basophilic lymphoid cells; this is in harmony with the opinion that the two cell types are related. The blood smear of Ma 796 and of Ma 760 suggested lymphomatosis, the sections atypical leukemia. These and the few other discrepant interpretations will have to be elucidated by further work. The present preliminary observations indicate the intricacy of the problem. The method of leukemogenesis used sets up a neoplastic and a nonneoplastic disturbance in different blood-forming systems varying greatly with different experimental animals. A bioassay of the blood during the early phases of leukemogenesis, with a more precise blood study, is in progress.

There is a discrepancy in the hematological and anatomic findings in mouse Ma 768. The blood smear clearly indicated leukemic lymphomatosis with lymphocytes of large, medium, and small size, and transitional forms. The granulocytes seen in the smear were of the mature type. The presence of numerous erythroblasts suggested bone-marrow involvement; but the section of the bone marrow showed myeloid hyperplasia with erythroblasts and only a few cells that were possibly of the lymphoid series. There was a marked periosteal infiltration, but a large number of cells in this location could be clearly recognized as myeloid. The liver showed advanced infiltration; the cells infiltrating the organ exceeded in bulk the liver tissue. Their character varied; some resembled lymphocytes, others atypical cells or myelocytes; there were occasional megakaryocytes and numerous mitotic figures. A few large areas of necrosis were noted in the spleen. The lymphoid foilicles were inconspicuous. The cells infiltrating the spleen were similar to those seen in the liver. The lymph node was densely infiltrated by similar cells, and there was a massive epicapsular infiltration. In the lung, a thick collar of similar cells surrounded the large vessels and the bronchi. In cross section of large veins, the cells present in the lumen were similar to those in the blood smear, although the relative number of polynuclears appeared to be higher in the section. In contrast with the cross section of large vessels, in which lymphocytes of small and medium size were numerous, the neoplastic cells forming the neoplastic "cuffing" were almost all large primitive cells with numerous mitotic figures. Thus, it is uncertain whether this mouse had leukemia of one type with marked secondary leukemoid disturbance or whether it had a mixed leukemia. Unfortunately, oxidase preparations are not available, and transmission experiments were not made.

DISCUSSION

The Onset of Induced Leukemia

The observations reported support the opinion that leukemia is not preceded by a long latency of malignant cells. Fischer (*19*) assumed that malignant cells are

present at birth but are inhibited from multiplication until at a later age some tissue derangement enables them to multiply and break through the restraining environment. This concept was contradicted by recent experiments (*14*) indicating that leukemic cells transmit the disease in very small numbers (10 or fewer) and do not remain latent in a new host. Subsequently, a difference was found between blood cells in spontaneous and experimentally induced leukemias (*11*). In spontaneous leukemias, potentially malignant cells resembling those of the pure leukemic ancestral cells may be present at birth, but not in a malignant form; they become malignant at a later age. In induced leukemias, normal cells become altered genetically and rendered malignant within from 2 to 4 months after application of the leukemogenic agent (*11*). The present experiments indicate that this transformation occurs rather suddenly during the seventh to thirteenth week of application of the carcinogen. Histologic evidence of malignancy at the early stage of the disease, demonstrable in transmission experiments, is scant and has been overlooked in three instances. Preceding this state, there is evidence of some derangement in lymphoid tissues, which are in most cases the primary sites of leukemia induced by methylcholanthrene. The germinal centers are larger than normal; mitotic figures are abundant; the pulp of the spleen is more cellular than normal pulp. A few cells present may resemble the malignant lymphocytes or atypical mononuclear cells, but leukemic blood cells do not have distinctive features to be identified with certainty, and the diagnosis of this disease in section is based on the presence of large numbers of abnormal cells at abnormal sites. The changes in mice during the first 7 weeks of painting resemble types of hyperplasia that may occur under different conditions. Millions of cells from the nodes and spleens of such mice introduced into young susceptible animals failed to produce leukemia. These nodes therefore contain a very few or no malignant cells. Then rather suddenly one cell type proliferates with great vigor, as a rule invades the blood stream, spreads throughout the lymphoid organs, and subsequently invades liver, bone marrow, and other organs, producing the characteristic changes of leukemia.

Type of Leukemia Induced by Methylcholanthrene

The 72 cases of leukemia produced in this series were classified as follows: lymphoid, 33; atypical, 29; myeloid, 2; monocytic, 2; untyped, 6. Thus the majority were either lymphoid or atypical. The malignant cells in this atypical form of leukemia resemble monocytes or large lymphocytes. Their cytoplasm is slightly basophilic and is free from granules. Their nuclei tend to become indented or lobed, and the convolutions formed are of a bizarre shape not seen in the usual myeloid cells. Doughnut-shaped nuclei characteristic of early myeloid cells of mice do not occur among them. These atypical leukemic cells were first observed in our laboratory (*17*) in a mouse that at 1 month of age received an intrasplenic injection of 1 mg. of benzpyrene. Lewis studied these cells in motion pictures and found that they moved like myeloid cells. Subsequently Lewis and Mider (*20*) studied a larger number of induced leukemias characterized by these atypical cells and confirmed a conclusion formerly reached by Lewis, namely, that the cells are myeloid in type.

A study of our present material indicates, however, that these atypical cells

occur in lymphoid tissues at a time when the bone marrow shows no leukemic proliferation and the liver is normal; hence they may well be lymphoid in origin; if the nuclear configuration is not conspicuous, they are not readily distinguished from large lymphocytes. It is conceivable that the atypical cells have arisen elsewhere and have metastasized in the lymph nodes, but the idea that they are myeloid in origin, with only hypothetical (unobserved) small foci of malignant cells in the bone marrow, followed by marked growth in the lymph nodes, contradicts experience with myeloid leukemias in all species. In myeloid leukemias the only consistent finding is advanced bone-marrow infiltration; changes in other organs (including blood changes) may be lacking. In mice of this series longitudinal cross sections of the femurs were examined (one or several cuts from each), but none of the slides of this series showed characteristic malignant cells at the early stage of the disease. Thus, the possibility that the atypical cells came from the bone marrow is most unlikely. This view is strongly supported by the lack of secondary manifestations of bone-marrow involvement, as anemia, thrombocytopenia, and by the lack of conspicuous qualitative changes in the blood picture of the myeloid series. On the other hand, the spleens of most mice of this series show nonmalignant myelopoiesis in the course of carcinogenesis, and myeloid leukemia may arise in these foci. It is conceivable, however, that the malignant cells came from the skin, where they were made malignant by the carcinogen applied to the skin but where they did not multiply to any extent. Infiltration of the skin by various types of blood cells is common in painted mice, but the skin is not as favorable a site for the multiplication of malignant blood cells as spleen, lymph nodes, and bone marrow. Malignant lymphocytes are found in blood smears 9 weeks after painting has begun, and a bioassay will probably show them at a still earlier time. Therefore, extreme caution is necessary to postulate any primary site of leukemic change on the basis of the available data.

Former studies have shown that the atypical cells exhibit no phagocytic activity and are invariably oxidase negative (*17*). The nuclear lobation is likewise not like that of cells in the known myeloid leukemias of mice. Cells with giant nuclei not seen in myeloid leukemias occur among these atypical cells.

On the basis of these data, it seems more reasonable to suppose that these atypical cells are not myeloid and that motility which characterizes the three main types of normal leukocytes (*20, 21*) should not be regarded as an entirely dependable means of diagnosing abnormal blood cells of leukemias. The small foci of these cells that were recognized and successfully assayed by transmission experiments occurred in the splenic pulp and in the reticulum meshes of lymph nodes and not in the lymphoid follicles; but leukemic lymphocytes may localize in such positions. Whether the atypical cells should be considered abnormal lymphocytes or monocytes or abnormal derivatives of the reticulum meshwork remains to be determined.

Three cases of granuloma of unidentified character with advanced leukemoid infiltration occurred among the painted mice. These proved not transmissible, although metabolically they resembled the leukemias. They will be described in another paper (*13*). The blood smears of these mice did not suggest leukemia.

Multicentric Origin of Leukemia

By analogy, it seems probable that the origin of leukemia is multicentric. The cutaneous tumors produced by painting

with methylcholanthrene are multicentric, and so are the lung tumors which occur in these mice. Hundreds, if not thousands, of adenomas are seen uniformly scattered throughout the lungs of mice painted with methylcholanthrene. These lung tumors do not show evidence of metastases at the early stage of the disease, and they seem to arise *in situ* from alveolar epithelium. Is it not likely that the lymphoid cells, too, are stimulated to malignant proliferation in numerous scattered foci?

The few instances of myeloid and of monocytic leukemia may have likewise been induced by the carcinogen, since all these occurred before the period of spontaneous leukemia in these stocks of mice. For the same reason, the nontransmissible granulomatous lesions can also be regarded as induced.

Thus, it seems that, when mice are painted with methylcholanthrene, carcinogenesis occurs in numerous organs and in numerous sites of the same organ. The method of application of the carcinogen and fortuitous circumstances determine which malignant change proceeds faster and brings about the death of the animal during the latent or early phase of different carcinomatous processes proceeding simultaneously. Most of our leukemic mice had multiple pulmonary and cutaneous tumors. Malignant blood cells, overwhelmingly either lymphoid or atypical but in several instances both types, were present. The absence of conspicuous myeloid proliferation in most is, however, noteworthy.

Influence of X-Rays on the Production of Leukemia by Methylcholanthrene

X-rays, as administered in our experiments, are leukemogenic (*15*, *16*), but after a much longer period than methylcholanthrene. The synergistic leukemogenic effects of X-rays and methylcholanthrene were studied for the following reason: X-rays injure the bone marrow and lymphoid organs; and when they are administered in a single large dose, destruction of these tissues is followed by a regenerative hyperplasia with numerous mitoses and often with atypical-cell formations. This tissue derangement may be expected to hasten the effect of another carcinogen administered at this stage and be productive of both myeloid and lymphoid leukemia.

Bioassay of Leukemia

The use of inbred mice has greatly enhanced the value of transmission experiments in demonstrating malignant cells. The current experiments show that in the diagnosis of leukemia it surpasses in sensitivity the usual diagnostic methods. It failed in only 1 of the 46 mice injected from 7 others when either gross or microscopic examination indicated leukemia. In 3 instances in which the gross and the microscopic examination were at first considered negative for leukemia and inoculation tests were positive, a review of the microscopic changes in lymph nodes indicated the presence of small foci of malignant cells. But, as already discussed, the histologic diagnosis of leukemia is not well-founded if it is based on small foci of atypical cells, and at the present time similar human cases can still not be regarded as definitely leukemic. Since autotransplantation is even more sensitive than homeotransplantation in inbred animals, this procedure may enable the determination of the malignant character of cells in different incurable diseases, e. g., in Hodgkin's disease, the nature of which is at present under dispute.

Mice Refractory to Induced Leukemia

Tables 1–4 show that a small number of mice which survived approximately 4

months of painting with methylcholanthrene and died at 6 months of age or later gave no evidence of leukemia. Whether these animals were refractory to the leukemia-producing agent used or whether they developed an immunity should be answered by further observations, although the former assumption seems highly probable.

SUMMARY

Leukemia was produced in mice by repeated percutaneous applications of methylcholanthrene. The animals were killed at intervals, blood-forming organs examined microscopically, and transmission experiments were made with suspensions of cells from spleen and lymph nodes.

Numerous transmission experiments made before the eighth week of painting were uniformly negative although hyperplastic changes were evident in lymphoid tissues. Three cases of leukemia were first demonstrated by transmission experiments. A subsequent microscopic review showed the presence of collections of atypical cells in the splenic pulp and in lymph nodes, while the bone marrow and liver showed no change. The numerous transmission experiments made from histologically definite cases of leukemia failed in only 1 of 46 injected mice. Of the tests used to determine the presence of leukemia, the gross examination is the least reliable, the microscopic examination next in order, and the inoculation experiments are the most decisive.

The type of leukemia produced was lymphoid or atypical, with the exception of a few myeloid or monocytic leukemias. Evidence is presented suggesting that the atypical cells originate in lymphoid tissues.

The onset of induced leukemia is sudden when judged by the criteria studied.

The incidence of leukemia was slightly higher among the X-rayed and painted mice than among the mice painted but not X-rayed.

A preliminary study of blood smears indicates that the majority of induced leukemias are accompanied by specific blood changes recognizable at least 2 weeks before the death of the animals.

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Metabolism of Induced and Spontaneous Leukemias in Mice [1]

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INTRODUCTION

The only studies that have been reported in the literature on the metabolism of leukemias in mice are the extensive investigations of Victor and coworkers (*2–11*) and of Hall and Furth (*12*) on lymph nodes of mice bearing spontaneous lymphatic leukemias or transmission lines derived therefrom. A wide variety of interrelated factors influencing the fermentation, respiration, and respiratory quotient of spontaneous and transmitted lines of leukemia was investigated by these workers, including age, sex, and strain (genetic constitution) of host mouse, reversibility of age and strain influences, host hybridization, period of time after inoculation in transmission experiments, effect of "leukemic" and "immune" sera as compared with normal serum or Ringer-bicarbonate solution, glucose concentration, and density of infiltration. The broad result obtained was that the metabolism of the lymph nodes of both the spontaneous and the transmitted leukemias examined showed a pronounced trend toward the metabolism of malignant tissues generally. Thus, possessing a respiratory quotient definitely less than unity, the leukemic lymph nodes developed a considerably increased rate of anaerobic and aerobic glycolysis (lactic and other acid production) as compared with normal lymph nodes. The metabolic distinction between the leukemic and normal lymph nodes was, however, quantitatively less striking than has been found to be the case for many human, rat, and chicken tumors when these have been compared with appropriate normal or homologous nonmalignant tissues (*13–16*). This was true in part because the normal lymph nodes of mice possessed, in contrast with many normal tissues, a very definite, though not large anaerobic and aerobic glycolysis.

The present paper reports studies on the metabolism of both myeloid and lymphoid

[1] *A brief summary* (1) *of this work was presented at the 35th annual meeting of the American Association for Cancer Research in Boston, in March 1942.*

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[3] *The Parker Research Fellowship was provided through the courtesy of Harvard University.*

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leukemias that have been induced in mice of known genetic constitution by treatment with methylcholanthrene alone or with methylcholanthrene and X-radiation combined. Determinations have been made not only upon lymph nodes but also upon spleens and livers, with results that add very considerably to an understanding of the general problems involved; also, manometric glycolysis values have in instances been checked by chemical methods. Comparisons have been made between normal mice, painted mice that were either preleukemic, leukemic, or leukemoid, and also mice inoculated with transmission lines of induced leukemia. Two cases of spontaneous leukemia were also measured, mainly as a basis for technical comparisons with results of other investigators on spontaneous leukemias.

Special attention has been devoted to ascertaining whether there is a definite preleukemic state that may be recognized from a metabolic point of view; in short, whether tissues show metabolic changes before they show unrestrained growth and invasiveness. By *preleukemic state* may be meant a transition period or condition that bears some definite or causal relationship to the leukemia that will develop later, and not merely a condition occurring prior to the leukemia but not inevitably followed by it. A study of leukemias induced at will by suitable leukemogenic agents provides an excellent opportunity to demarcate more rigidly than heretofore the period or condition in the life span of an animal that might properly be called the preleukemic state and that inevitably leads to the leukemic state, irrespective of the original leukemogenic agent.

The details on aspects of the animal experimentation and pathologic examinations are described in the accompanying paper (*17*), the metabolic observations in this paper. The metabolic results obtained in two different, consecutive series of experiments reported were not always as conclusive as might be desired, because of insufficient repetition or confirmation, circumstances not permitting continued, more thorough investigation.

METHODS

Two stocks of mice have been studied, Rf in which the spontaneous incidence of leukemia is low (3 percent), and the F_1 hybrid Rf/Ak in which the incidence is 12 percent (the incidence in the Ak parent stock being 70 percent). Three-to-six-weeks old mice were painted with 0.5 percent methylcholanthrene in benzol twice a week at alternating sites, litter mates being used for unpainted controls. Mice were killed after varying intervals of time and their tissues examined grossly and microscopically for the presence of leukemia. Fragments of spleen and lymph nodes were minced under sterile conditions in Tyrode's solution and, after filtering, 0.1-cc. portions were injected intravenously into healthy mice of the same strain in an attempt at bioassay of leukemia. Such transmission experiments are especially suitable for determining early leukemias that might escape diagnosis on gross or microscopic examination, in addition to confirming gross and microscopic diagnoses, positive or negative. Some of the animals painted with methylcholanthrene were also X-irradiated with 300 r, either 1 day before or 2 weeks after commencement of the painting regime, in order to note possible effects that might be obtained upon combined use of these two types of leukemogenic agents. Experiments with X-irradiation alone were not carried out in this investigation.

Complete details regarding the methods and procedure for obtaining metabolic values, together with considerations of many special technical problems, including sources of error and interpretation of

replicate variability, were described previously in this journal (*16*). By means of the recently developed Summerson constant-volume differential manometer (*18*), the ordinary simple constant-volume Warburg manometer, and the recently developed method of Barker and Summerson (*19*) for determining lactic acid chemically, the following standard metabolism determinations were carried out on tissues thermostated at 38° C.: aerobic acid production ($Q^{O_2}_A$), respiratory carbon dioxide production (Q_{CO_2}), oxygen consumption (Q_{O_2}), and respiratory quotient ($R.Q=Q_{CO_2}/Q_{O_2}$), all for a 2-hour experimental period; and anaerobic acid production ($Q^{N_2}_A$), ordinarily for 4 hours by hourly periods; and the chemical values, aerobic and anaerobic lactic acid production ($Q^{O_2}_{LA}$) and ($Q^{N_2}_{LA}$). All Q values are reported in terms of cubic millimeters/milligram initial dry weight/hour, whereby 1 $mm.^3 = 1/22400000$ mol. Initial, rather than final dry-weight determinations have been employed in every instance. In the cases of spleen and liver the measured initial dry weights were employed; but in the case of lymph nodes not infrequently very little material was available for making suitable determinations, and although the initial dry-weight values are reported, all node values were arbitrarily calculated upon an assumption of 18 percent dry weight of initial (accurately measured) wet weights taken for determination and reported in the tables. We feel that this procedure is much more satisfactory on the whole than to use the particular dry weights measured, since it is very likely that particularly high or low measured values obtained were less accurate than the assumed ones. The measured values are recorded, however, in the event that future experimentation indicates that values above 25 percent initial dry weight or below 15 percent, for the techniques employed, really occur. Practically all the values of other previous investigators (cited later in this paper) are based upon an assumed initial dry weight of 19.1 (*12*) or 20 (*2–11*) percent of the measured wet weight, the actual initial dry weights having seldom been measured. The present procedure in assuming a percentage dry weight in the case of lymph nodes is thus consistent with that of others; for our conditions 18 percent initial dry weight appeared to be nearer the most probable average than 19 or 20 percent.

RESULTS

The metabolism measurements were carried out in two series, the first series on stock Rf studied in 1940, described as experiment 1 in the preceding paper (*17*), and the second series on stock Rf/Ak studied in 1941 and referred to (*17*) as experiments 2, 3, and 4. The second series is here described first, inasmuch as it involved only anaerobic measurements, and the results obtained were more consistent and conclusive. The anaerobic glycolysis or fermentation is probably the most important single metabolic property of interest in connection with the metabolism of malignant cells. Although by no means specific therefor, it is, so far as is known, an invariable characteristic thereof, which is not always true of aerobic fermentation. The experimental anaerobic glycolysis values reported in the tables are given hourly through the fourth hour in order to indicate any changes of glycolysis with time. As discussed earlier (*16, p. 225*) the 4-hour average $Q^{N_2}_A$ values are probably as, or more, deserving of consideration than the first or any other hour. In examining the metabolic data it is best to focus attention first on the average values; in general they most nearly equal the second-hour values.

Stock Rf/Ak

The experimental results with the stock Rf/Ak are presented in table 1, which contains the metabolic values for lymph nodes, spleen, and liver of unpainted controls, painted preleukemic mice, and painted leukemic mice. The first leukemic mice metabolized were obtained 9 weeks after painting with methylcholanthrene and X-irradiation with 300 r 2 weeks after commencement of the painting, both lymphoid (L) and atypical (A) types being obtained. A monocytic (Mo) form was obtained at 10 weeks after painting and irradiation, and a myeloid (M) type after 14–15 weeks. Nonmalignant extramedullary myelopoiesis (NEM) was observed in various instances, particularly in leukemic spleens, but also in preleukemic and even control spleens. Much hyperplasia was noted in lymph nodes and spleens of preleukemic mice.

Lymph Nodes and Spleen

A remarkable metabolic result obtained was that with both Rf/Ak lymph nodes and spleens there was no evident difference in average anaerobic glycolysis between the control, preleukemic, and leukemic groups. This was true irrespective of the type or extent of infiltration in the leukemic group. There was, to be sure, a considerable range of variation in the average $Q^{N}_{2_A}$ values ($Q^{N}_{2_A}$=4–9 mm.^{3}/lactic or other acid formed per hour per milligram initial dry weight) in all three groups, control, preleukemic, and leukemic. There also appears to be some trend for the glycolysis values to increase with age of animal, although this is not definite. No marked effect of sex of animal upon the metabolic values is evident, although the data are scarcely suitable for throwing light on this point. There are a few relatively high $Q^{N}_{2_A}$ values for leukemic node and spleen (thus Ma 768 node and Mb 5 spleen, and Mb 5 liver) for which we hesitate to assign any specific cause. The inclusion of monocytes in the Mb 5 spleen and liver is suggestive in this connection, although unpublished data of Hall (cited in table 4) do not indicate high values for the malignant monocytic cells he studied. Possibly the combination of monocytic and atypical cells leads to high values. The fact that the Mb 5 mouse had been leukemic for at least 17 days before metabolic analysis may be the chief factor. The reason for the discrepancy between replicate determinations of the preleukemic lymph nodes of February 12 cannot be assigned, but we would be inclined to regard the lower replicate as the more probable.

In view of the considerable variation of metabolic values observed in all three groups, a study of a much greater number of animals in each group might have revealed some small statistically significant difference between the groups of say 20 percent or less. However, this would be of very doubtful interest, and might merely reflect some quite incidental, technical aspect, such as a slightly different mode of slicing, depending upon size of organ, a fortuitous average animal age difference, etc. The absence of glycolytic increase in the lymph nodes of the induced Rf/Ak leukemias found here is in striking contrast with all previously reported cases of spontaneous leukemia, where the average glycolysis values commonly increased twofold and often more as compared with comparable normal nodes. A similar lack of metabolic distinction between spleen or bone marrow of normal and erythroleukotic chickens has been briefly reported before (*22*). The lack of distinction between normal and malignant cells in the case of leukemic lymph nodes and spleen comes about from the two circumstances that the anaerobic glycolysis values of

TABLE 1.—*Anaerobic metabolism of lymph nodes, spleen, and liver of normal, preleukemic, and leukemic stock Rf/Ak mice*

Animal No. and sex [1]	Date	Time after painting [2]	Diagnosis: Gross	Diagnosis: Microscopic	Diagnosis: Type [3]	Tissue: Lymph nodes: Node type if particular [4]	Dry weight (initial)	Wet weight	$Q^{N_2}_A$ Hour: 1st	2nd	3rd	4th	Average	Ratio 4th/1st hour	Degree and type [5] of infiltration
Unpainted, controls:	*1941*	*Weeks*					*Pct.*	*Mg.*							
Mb: 52m, 58m, 60f, 70m	Mar. 22	(7)	−	−			22	59 7.4	6.8 7.0	5.9 4.2	4.7 4.4	3.7 2.9	5.3 4.6	0.54 .42	0
Ma: 950f, 784m, Mb: 8f	Feb. 19	(9)	−	−			27	29	8.9	8.3	6.4	5.9	7.4	.66	0
Mb: 89f, 91f, 97f, 99m	May 9	(13)	−	−			16	50 37	9.1 8.5	8.2 8.0	7.2 7.1	6.6 6.4	7.8 7.5	.73 .75	0
Average		(9.7)					21.7	36.5	8.1	6.9	5.9	5.1	6.5	.63	
Painted, negative (preleukemic): Ma: 933f, 936f, 941f, 946m, 951m, 956f, 961m.	Feb. 12	4	−	−			(36)	26 49	13.6 7.2	11.2 3.1	9.4 3.4	8.4 3.5	10.6 4.3	.62 .49	0 (hyperplasia)
Mb: 57m, 59m, 65m, 77m	Mar. 22	7	−	−			(39)	88 77	7.8 4.1	5.6 3.9	5.2 3.2	3.7 2.6	5.6 3.5	.47 .63	0 (65, slight hyperplasia).
Mb: 88f, 90m, 96f	May 9	13	−	[7] −			(13)	48 42	9.6 5.9	9.1 7.8	8.0 5.3	7.3 6.2	8.5 6.3	.76 1.05	[7] 0
Average		8					(29)	55	8.0	6.8	5.7	5.3	6.5	.67	
Painted, leukemic: Ma: 762m [8]	Feb. 19	[9] 9	−	+	L		32	34	7.5	7.0	6.2	5.6	6.6	.75	2L
Ma: 750m	Feb. 19	[9] 9	+	+	A		30	34 35	8.1 7.6	6.7 4.0	5.0 3.0	3.4 2.6	5.8 4.4	.42 .34	3A (some NEM)
Ma: 796f	Apr. 5	12	+	+	A	a. c, i.	21 23	49 38	9.5 8.9	7.7 7.1	4.3 5.0	2.5 3.0	6.0 6.0	.26 .34	3A
Ma: 810m	Apr. 5	[10] 12	+	+	L		93	93	6.9	6.4	4.4	3.6	5.3	.52	3L
Ma: 767m	Mar. 22	13	+	+	L	a. c. i.	25 20 22	50 76 89	8.8 7.0 5.4	7.6 5.5 4.3	6.1 4.3 3.1	4.3 2.9 2.2	6.7 4.9 3.8	.49 .41 .41	3¼L
Mb: 5f	Apr. 5	[10] 10	+	+	Mo–A	c, i. a.	17 19	33 33	7.6 7.2	6.5 5.7	5.5 4.6	4.9 4.6	6.1 5.5	.65 .64	2A (Mo, 25 pct.)
Ma: 768f	Apr. 5	[9] 15	+	+	M	a. c, i.	21 20	46 103	13.0 10.8	10.1 9.7	8.0 7.5	6.3 6.2	9.4 8.6	.49 .57	3M
Mb: 49f	May 9	14	+	+	M–A [11]		19	39 37	9.1 9.1	8.4 8.3	7.7 7.7	6.7 6.7	8.0 8.0	.74 .74	2M–A [11]
Average		12					21.6	52.6	8.4	7.0	5.5	4.4	6.3	.52	

Tissue: Spleen: Dry weight (initial)	Wet weight	$Q^{N_2}_A$ Hour: 1st	2nd	3rd	4th	Average	Ratio 4th/1st hour	Degree and type [5] of infiltration
Pct.	*Mg.*							
20.3	112	7.3	4.8	3.0	1.1	4.0	0.15	0 (58, 60, 1½ NEM) [6]
24.1	46 58	5.3 4.9	5.2 4.4	4.4 3.3	3.5 3.0	4.6 3.9	.67 .61	0
20.2	37 96	6.9 7.0	6.0 5.9	5.3 5.2	4.8 4.5	5.7 5.6	.70 .64	0
21.5	70	6.3	5.3	4.2	3.4	4.7	.55	
23.7	26 42	8.4 5.4	6.9 4.2	6.4 4.0	5.4 3.3	6.8 4.2	.64 .61	0 (hyperplasia)
20.0	78	6.1	4.4	3.6	1.8	[6] 4.0	.30	0 (1 NEM)
19.2	39 76	8.8 6.3	5.4 5.4	5.9 5.6	5.1 3.9	6.4 5.3	.58 .62	0 (2 NEM)
20.9	52	7.0	5.3	5.1	3.9	5.3	.55	
25.6	26	5.7	4.6	4.4	3.6	4.6	.63	2L (1½ NEM)
22.4	94 90	7.5 6.2	7.5 2.2	3.2 1.2	2.4 .3	5.1 2.5	.32 (.05)	2½A (1 NEM)
20.4	28	5.4	3.9	2.8	3.5	3.9	.65	3A
20.4	33	9.1	7.5	6.4	5.2	7.0	.57	2L (NEM)
30.6	52	5.4	4.7	3.7	2.4	4.1	.44	4L
20.4	24	13.5	11.2	10.0	6.5	10.3	.48	1½A (1 Mo, 2½ NEM)
20.4	32	9.7	7.5	6.6	2.9	6.7	.30	2M
12.7	59 56	6.5 4.7	6.1 4.9	5.3 4.4	4.6 3.7	5.6 4.4	.71 .79	3A–M [11]
21.6	49.4	7.4	6.0	4.8	3.5	5.4	.50	

[1] Sex indicated by letter m (male) or f (female) after number of animal.

[2] Time after painting at 4–5 weeks: parenthetical values are for unpainted control mice of equivalent age, calculated from 4–5 (not 0) weeks.

[3] L signifies lymphoid leukemia, M myeloid, Mo monocytic, and A atypical. The atypical leukemia is similar in distribution to lymphoid leukemia, but the cells are not typical lymphocytes and their identity is uncertain (Barnes and Furth (*20*)). The degree of infiltration is a semi-quantitative grading between 0 and 4 where 4 represents the maximum ordinarily ever observed. In liver the grades 1, 2, and 3 correspond to an estimated 10, 20, and 30 percent of infiltrated cells among liver cells, and grade 4 means any infiltration of 40 percent or greater.

[4] a, axillary; i, inguinal; c, cervical. 4 mm. is normal for cervical, but is markedly enlarged for other superficial nodes. The deep nodes are seldom enlarged.

[5] NEM signifies nonmalignant extramedullary myelopoiesis indicated by the presence of erythroblasts and myelocytes (including polymorphonuclear leukocytes, eosinophiles in various stages of maturity, and occasional megakaryocytes (Barnes and Sisman (*21*)).

[6] Q_{O_2}, Q_{CO_2}, Q_{O_2}, and R. Q. were, respectively, 1.43, 1.94, 4.15, and 0.4[illegible] for spleen, and 0.56, 1.[illegible] some admixture with the Mb 88 node thought to be negative at the time of metabolic analysis, but May 9.)

[7] In Mb 88, some hyperplasia in nodes, and mediastinal node with high[illegible]-degree, malign[illegible]

[8] Transmission test positive. No other metabolized mice of this series were tested, although accompanying paper (*17*).

[9] Irradiated with 300 r 2 weeks after painting commenced.

[10] Irradiated with 300 r 1 day before painting commenced.

[11] These cells not identified with certainty; they may be myeloid or a [illegible] of both (mixed

lymph nodes, spleen, and liver of normal, preleukemic, and leukemic stock Rf/Ak mice

Tissue																	
		Spleen															
				$Q^{N_2}{}_A$													
				Hour													
Ratio 4th/1st hour	Degree and type [1] of infiltration	Dry weight (initial)	Wet weight	1st	2nd	3rd	4th	Average	Ratio 4th/1st hour	Degree and type [2] of infiltration	Dry weight (initial)	Wet weight	1st	2nd			
		Pct.	*Mg.*								*Pct.*	*Mg.*					
0.54 .42	0	20.3	112	7.3	4.8	3.0	1.1	4.0	0.15	0 (58, 60, 1½ NEM) [3]	22.5	147 127	0.66 .84	0.62 .44			
.66	0	24.1	46 58	5.3 4.9	5.2 4.4	4.4 3.3	3.5 3.0	4.6 3.9	.67 .61	0	22.7 25.9	185 193 249	.76 .72 .54	.53 .40 .33			
.73 .75	0	20.2	37 96	6.9 7.0	6.0 5.9	5.3 5.2	4.8 4.5	5.7 5.6	.70 .64	0	18.0	149 149	.61 .46	.38 .25			
.63		21.5	70	6.3	5.3	4.2	3.4	4.7	.55		22.3	171	.65	.42			
.62 .49	0 (hyperplasia)	23.7	26 42	8.4 5.4	6.9 4.2	6.4 4.0	5.4 3.3	6.8 4.2	.64 .61	0 (hyperplasia)	23.8	101	.90	.70			
.47 .63	0 (65, slight hyperplasia).	20.0	78	6.1	4.4	3.6	1.8	[8] 4.0	.30	0 (1 NEM)	20.1	169 121 136 122	.81 .96 .53 .54	.63 .60 .38 .43			
.76 1.05	0 [7]	19.2	39 76	8.8 6.3	5.4 5.4	5.9 5.6	5.1 3.9	6.4 5.3	.58 .62	0 (2 NEM)	15.2	155 159	.65 .68	.64 .51			
.67		20.9	52	7.0	5.3	5.1	3.9	5.3	.55		19.7	137	.72	.56			
.75	2L	25.6	26	5.7	4.6	4.4	3.6	4.6	.63	2L (1½ NEM)	24.0	200 242	1.7 1.8	.82 .78			
.42 .34	3A (some NEM)	22.4	94 90	7.5 6.2	7.5 2.2	3.2 1.2	2.4 .3	5.1 2.5	.32 (.05)	2½A (1 NEM)	23.0	131 255	1.6 1.9	1.6 1.5			
.26 .34	3A	20.4	28	5.4	3.9	2.8	3.5	3.9	.65	3A	25.0	209	2.7	2.0			
.52	3L	20.4	33	9.1	7.5	6.4	5.2	7.0	.57	2L (NEM)	25.0	199	3.1	2.4			
.49 .41 .41	3½L	30.6	52	5.4	4.7	3.7	2.4	4.1	.44	4L	19.5	143	3.3	1.3			
.65 .64	2A (Mo, 25 pct.)	20.4	24	13.5	11.2	10.0	6.5	10.3	.48	1½A (1 Mo, 2½ NEM)	20.5	188 209	6.1 5.6	4.3 3.7			
.49 .57	3M	20.4	32	9.7	7.5	6.6	2.9	6.7	.30	2M	20.8	194 209	5.4 5.9	4.2 4.2			
.74 .74	2M–A [11]	12.7	59 56	6.5 4.7	6.1 4.9	5.3 4.4	4.6 3.7	5.6 4.4	.71 .79	3A–M [11]	18.4	154 150	6.9 6.2	5.1 4.7			
.52		21.6	49.4	7.4	6.0	4.8	3.5	5.4	.50		22.0	191	4.0	2.8			

weeks.
mphoid leukemia, but the
ve grading between 0 and
percent of infiltrated cells

odes are seldom enlarged.
polymorphonuclear leuko-

[6] $Q^{O_2}{}_A$, Q_{CO_2}, Q_{O_2} and R. Q. were, respectively, 1.43, 1.94, 4.15, and 0.47 for spleen, and 0.56, 1.44, 2.5, and 0.6

[7] In Mb 88, some hyperplasia in nodes, and mediastinal node with high, fourth-degree, malignant, lymphoid
some admixture with the Mb 88 node thought to be negative at the time of metabolic analysis, but evidently w
May 9.)

[8] Transmission test positive. No other metabolized mice of this series were tested, although many not met
accompanying paper (*17*).

[9] Irradiated with 300 r 2 weeks after painting commenced.

[10] Irradiated with 300 r 1 day before painting commenced.

[11] These cells not identified with certainty; they may be myeloid or atypical or both (mixed M or A).

Liver

$Q^{N_2}{}_A$ Hour						Degree and type of infiltration
1st	2nd	3rd	4th	Average	Ratio 4th/1st hour	
0.66	0.62	0.57	------	0.62	------	0.
.84	.44	.56	------	.61	------	
.76	.53	.43	0.32	.51	0.42	0.
.72	.40	.40	.28	.45	.39	
.54	.33	.39	.36	.41	.67	
.61	.38	.46	.30	.44	.49	0.
.46	.25	.46	.30	.37	.65	
.65	.42	.47	.31	.49	.52	
.90	.70	.49	.44	.63	.49	0.
.81	.63	.68	------	[6].71	------	0.
.96	.60	.42	------	.66	------	
.53	.38	.48	------	.46	------	
.54	.43	.39	------	.34	------	
.65	.64	.64	.55	.62	.85	0 (88, 1L).
.68	.51	.64	.55	.59	.81	
.72	.56	.53	.51	.57	.71	
1.7	.82	.61	.52	.94	.31	¾L (¾ NEM).
1.8	.78	.54	.47	.89	.26	
1.6	1.6	1.1	.8	1.3	.50	1¼ A.
1.9	1.5	1.0	.65	1.2	.34	
2.7	2.0	1.1	.8	1.7	.30	1¾A.
3.1	2.4	1.6	1.0	2.0	.32	2L.
3.3	1.3	1.7	------	2.1	------	3L.
6.1	4.3	2.0	1.2	3.4	.19	3 Mo (1A).
5.6	3.7	1.7	.9	3.0	.16	
5.4	4.2	2.7	1.8	3.5	.33	4 M.
5.9	4.2	2.4	1.5	3.4	.25	
6.9	5.1	3.3	1.8	4.3	.26	2½ M–A [11].
6.2	4.7	2.8	2.6	4.1	.42	Some giant cells.
4.0	2.8	1.7	1.2	2.4	.30	

, 2.5, and 0.61 for liver.

t, lymphoid infiltration; the metabolic values reported involve
evidently without noticeable effect. (Cf. unpainted controls,

any not metabolized were tested with results described in the

or A).

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normal lymph nodes and spleens represent with few exceptions about the upper limit for stable adult animal tissues generally and about the lower limit of anaerobic glycolysis for malignant cells. Metabolic characterizations other than anaerobic glycolysis might well show differentiation between normal and malignant node and splenic tissue.

Liver

That malignant cells infiltrating leukemic nodes and spleens do have a definite anaerobic glycolysis that is not obscured by the bulk of normal node and splenic tissue containing them is strikingly brought out by the metabolic observations on liver reported in table 1 and reproduced in part graphically in figure 1. Normal liver, in contrast with node and spleen, is seen to possess a very low, almost negligible anaerobic glycolysis. The preleukemic values are no greater or only slightly so. With leukemic liver, however, the anaerobic glycolysis increased immediately and simultaneously with the histopathologic indication of onset of disease and was correlated with eventual successful transmission. The average anaerobic glycolysis values rose from about 0.5 to 1 to 4, or a twofold to eightfold increase, depending very closely upon the extent of infiltration as observed by histologic examination carried out independently and without knowledge of the metabolic values. Figure 1 brings out the correlation (approximate proportionality) between glycolysis and extent of infiltration. The type of leukemic infiltration appears to be of relatively little importance compared with the extent of infiltration, except for the bare suggestion (also borne out in spleen as well as in liver) that the monocytic cells have somewhat greater values than the other leukemic types observed. Unfortunately, none of the control or preleukemic livers contained nonmalignant extramedullary myelopoiesis, so that it was impossible in the Rf/Ak series to determine the metabolic characteristics of this type of infiltration. It is evident that liver, because of its very low intrinsic glycolytic value, is in general an excellent organ with which to determine the approximate anaerobic glycolytic rate of various types of infiltration. This might not be so true of aerobic glycolysis since under aerobic conditions liver might well utilize the glycolytic products formed (e. g., lactic acid) and so mask measurement of their production.

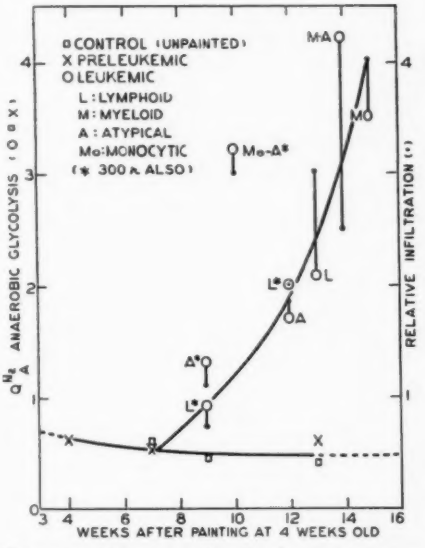

Figure 1.—The proportionality between anaerobic glycolysis and infiltration in leukemic livers of stock Rf/Ak mice. Vertical lines connect respective metabolic (o) and infiltration (.) values.

At any rate, as is evident in table 1, liver is a far better organ to detect early leukemic or even preleukemic changes when they occur than are lymph nodes or spleen, in view of the vastly greater difference between normal and leukemic states, and

hence much greater opportunity for differentiation between preleukemic and either normal or leukemic states.

Time Course

In the foregoing considerations of anaerobic glycolysis values, the change in anaerobic glycolysis with time is a matter of some interest. The column "ratio 4th to 1st hour" provides a measure of the decrease with time. In general, for all three types of tissues studied the decrease in glycolytic rate is 40 to 50 percent by the fourth hour of observation. The decrease is, in fact, slightly greater for the leukemic rather than nonleukemic tissues, which is somewhat surprising in view of the fact that with most tumor tissues (*16, p. 225*) the decrease in anaerobic glycolysis with time is markedly less than in most nonmalignant tissues. Leukemic cells are generally more fragile than comparable normal blood cells. The slightly greater decrease in glycolytic rate with time, in the case of all three leukemic tissues studied as compared with the corresponding nonleukemic tissues, is one more indication that mouse leukemic-tissue metabolism, as well as the leukotic chicken-tissue metabolism already referred to, is probably as little characteristic of malignant cell metabolism as any observed by investigators so far, with respect to the glycolytic criterion under consideration.

Study of the several metabolic values in table 1 involving either X-irradiation or nonmalignant extramedullary myelopoiesis suggests no notable metabolic influences of these factors.

Stock Rf

The experimental results with stock Rf are presented in table 2, which provides, in addition to results of the type given in table 1, manometric determinations for aerobic as well as anaerobic conditions, observations on two transmitted leukemias (second subtransfers from leukemias produced by methylcholanthrene painting), some observations on two cases of nontransmissible leukemoid infiltrations, and also, for technical comparison, two cases of spontaneous lymphoid leukemias in stock Ak–C3H. The three last-mentioned miscellaneous groups were not studied in numbers sufficient to permit much generalization, and indeed both the transmissible and leukemoid groups yielded within themselves widely varying values that call for further work. In the Rf series reported in table 2, all the induced leukemias metabolized were lymphoid, although the spontaneous leukemias characteristic of this stock are monocytic, or myeloid. The first case of leukemia in the Rf mice that were obtained for metabolism developed after 16 weeks of painting, except for the single mouse (Rf 150, lymph nodes only) in which leukemia appeared at 12 weeks, and which was unrecognized as leukemic at the time of metabolic analysis and so was analyzed together with two nonleukemic (preleukemic) mice. The results in table 2 are less uniform and conclusive than those in table 1 and are in certain respects better regarded as an initial survey.

Anaerobic Glycolysis

With few exceptions, conclusions on anaerobic metabolism drawn from table 1 are supported in table 2, where the data are adequate. Both tables indicate that the preleukemic or leukemic nodes and spleens do not possess an anaerobic glycolysis that is different from that of the unpainted controls. However, in view of the low anaerobic values in two of the four sets of unpainted Rf control animals, this conclusion is from a statistical point of view perhaps somewhat less certain, especially

TABLE 2.—*Aerobic and*

Animal No. and sex	Date	Time after painting [1]	Diagnosis			Lymph nodes											
			Gross	Microscopic test	Transmission test	Dry weight (initial)	Wet weight		Aerobic				Anaerobic				
							Aerobic	Anaerobic	$Q^{O_2}_A$	Q_{CO_2}	Q_{O_2}	R. Q.	$Q^{N_2}_A$				
													Hour				
													1st	2d	3d	4th	Average
Unpainted, contro s:	*1940*	*Weeks*				*Pct.*	*Mg.*	*Mg.*									
156m, 157m, 158m, 159m	Sept. 30	(14)	–	–	.....	22.6	78	15	2.5	2.4	2.9	0.83	3.3	3.3	2.6	2.0	2.8
								17					2.4	1.4	1.5	1.4	1.7
164m, 165f, 166m, 167f	Oct. 7	(16)	–	–	.....	27.8	75	20	2.3	2.5	2.8	.89	3.5	3.0	3.0	3.0	3.1
								20					3.2	3.0	3.0	3.0	3.0
269m, 270m, 271m, 272m, 273m, 274m	Dec. 11	(16.5)	–	–	.....	(35.5)	75	32	2.2	3.3	4.7	.78	7.8	7.6	6.7	5.9	7.0
								31					7.1	6.1	5.5	3.1	5.4
260m, 261m, 262f, 263f, 264m	Dec. 5	(23.5)	–	–	.....	[4] 17.7	65	23	1.4	3.6	4.6	.78	7.2	9.9	6.9	6.9	7.7
								25					6.7	5.4	5.0	5.7	5.7
Average		(17.5)	.....	.....	.....	25.9	73	23	2.1	2.9	3.7	.82	5.3	4.9	4.3	3.9	4.6
Painted, negative (preleukemic):																	
124m, 125m, 126f	Aug. 16	7	–	–	[5]	20	76	43	.....	3.1	3.9	.80	5.4	6.1	6.3	5.2	5.7
								44					4.9	5.3	5.4	4.3	5.0
129f, 130m, 131f	Aug. 23	8	–	–	[5]	15	76	25	2.9	4.0	4.8	.84	7.5	6.6	6.3	5.9	6.6
								25					7.3	5.6	5.3	4.9	5.8
134f, 135f, 136m	Aug. 30	9	–	–	[5]	15	76	18	3.0	2.4	3.7	.66	6.0	5.0	5.3	4.5	5.2
								18					4.5	5.3	5.1	4.5	4.8
137m, 138f, 139m	Sept. 6	10	–	–	[5]	16	63	16	2.9	2.1	3.1	.69	5.0	4.8	4.6	.....	4.8
								15					4.6	4.6	4.4	.....	4.5
144f, 145m, 146m	Sept. 13	11	–	–	[5]–	16	60	15	3.7	2.1	2.8	.77	5.3	4.2	4.0	4.8	4.6
								15					3.8	3.5	3.5	3.3	3.5
149f, 150m, 151f	Sept. 20	12	[5]±	[5]±	[5]±	17	75	16	3.2	1.5	1.9	.80	7.5	7.5	6.1	5.6	6.7
								16					6.1	6.4	4.7	3.3	5.1
152m, 153f, 154m	Sept. 27	13	–	–	[5]–	17	103	17	2.8	2.3	2.7	.83	5.3	5.0	4.7	4.5	4.9
								18					4.4	4.7	3.7	3.7	4.1
160m, 161m, 162f	Oct. 4	14	–	–	[5]–	15	61	17	2.4	4.2	4.4	.94	7.6	7.8	7.6	7.6	7.6
								19					6.3	6.3	5.9	6.5	6.2
169f, 170f, 171m	Oct. 11	15	–	–	[5]–	18	75	16	.....	.....	3.7	.84	6.6	6.8	5.8	6.0	6.3
													5.1	5.9	4.6	4.8	5.1
Average		11	.....	.....	.....	16.6	73	20	3.0	2.7	3.4	.80	5.5	5.6	5.2	5.0	5.3
Painted, leukemic:																	
212f	Oct. 18	16	+	+	+	[16	103	20	2.9	3.5	4.9	.70	9.9	8.1	5.5	2.8	6.6
							103	17	2.7	3.1	4.5	.69	8.6	7.3	4.7	3.6	6.0
						[15	102	20	2.8	4.0	4.7	.84	6.5	5.4	3.2	2.7	4.5
							103	20	2.7	3.3	3.7	.89	5.3	3.7	2.6	2.4	3.5
222m	Oct. 23	17	+	+	+	15	45	.....	4.0	3.2	4.8	.74	.....	.....	.....	.....	.....
246m	Nov. 20	21	+	+	+	21	76	16	2.6	5.1	6.0	.86	6.7	5.5	3.9	3.4	4.9
								15					6.3	3.6	4.3	2.7	4.2
Average		18	.....	.....	.....	16.8	89	18	3.0	3.7	4.8	.79	7.2	5.6	4.0	2.9	4.9
Transmitted (unpainted):																	
240f, 241f (2d transfer from 150; inoculated Oct. 11, 1940).	Nov. 4	(3)	+	+	+	21	76	17	3.7	4.3	4.0	(1.06)	9.8	5.4	3.5	1.8	5.1
								17	.....	.....	.....	.....	8.2	4.5	3.0	1.7	4.4
265m (2d transfer from 212; inoculated Nov. 26, 1940).	Dec. 7	(1½)	+	+	+	16.7	76	30	2.3	4.4	5.9	.75	.....	12.6	7.1	6.9	8.9
								28	.....	.....	.....	.....	.....	11.0	6.3	6.5	7.9
Average																	

erobic and anaerobic metabolism of lymph nodes, spleen, and liver of control, preleukemic, leukemic, and leukemoid stock Rf mice

Tissues

					Spleen																	
						Wet weight		Aerobic				Anaerobic										
												$Q^{N_2}{}_A$										
												Hour										
	A verage	Ratio 4th/ 1st hour	Node size (axillary or inguinal) (largest diameter)	Degree and type [2] of infiltration	Dry weight (initial)	Aerobic	Anaerobic	$Q^{O_2}{}_A$	Q_{CO_2}	Q_{O_2}	R. Q.	1st	2d	3d	4th	A verage	Ratio 4th/ 1st hour	Spleen size in diameters	Degree and type [2] of infiltration			
			Mm.		*Pct.*	*Mg.*	*Mg.*											*Mm.*				
0	2.8	0.61	2	0	17.5	76	17	3.6	2.5	3.3	0.76	4.1	4.1	1.3	1.8	2.8	0.44	14 x 5	0			
4	1.7	.58					15					3.7	3.4	2.8	2.2	3.0	.59					
0	3.1	.86	2	0	20.0	75	20	3.6	2.3	3.6	.64	5.2	5.0	4.5	4.5	4.8	.87	15 x 4	0			
0	3.0	.94					21					5.3	5.0	4.4	4.4	4.8	.83					
9	7.0	.76	2	0	18.0		30					8.1	6.4	5.8	5.4	6.4	.67	15 x 5	0 (1 NEM)			
1	5.4	.44					32					6.6	5.7	5.3	4.0	5.4	.61					
9	7.7	.96	2	0	19.3	64	22	2.4	3.9	5.3	.74	7.4	7.8	6.9	6.7	7.2	.90	15 x 5	0 (1 NEM)			
7	5.7	.85					21					6.1	7.6	6.9	6.8	6.8	1.07					
9	4.6	.74	2		18.7	72	22	3.2	2.9	4.1	.71	5.8	5.6	4.7	4.4	5.1	.76	14.7 x 4.7				
2	5.7	.96	3.5	0														16 x 6	0			
3	5.0	.88																				
9	6.6	.79	3.5	0														16 x 5	0			
9	5.8	.67																				
5	5.2	.75	3.5	0	15	28	15	5.2	2.0	4.6	.44	7.7	6.0	5.3	5.5	6.1	.71	15 x 5	0			
5	4.8	1.00					17					6.5	5.8	5.5	5.2	5.8	.80					
---	4.8	-----	3.5	0	16	25	15	6.6	3.2	6.4	.50	7.4	7.1	7.4	-----	7.3	-----	14 x 5	0			
---	4.5	-----					15					4.7	4.4	4.7	-----	4.6	-----					
8	4.6	.91	3.5	0	18	29	15	4.3	4.9	6.2	.79	9.1	6.2	7.0	6.8	7.3	.75	15 x 5	0 (3 NEM)			
3	3.5	.87					15					9.1	5.9	7.6	5.6	7.1	.62					
6	6.7	.75	3.5	0	17	18	14	7.2	2.2	5.4	.41	4.7	5.1	4.0	3.4	4.3	.72	15 x 5	0			
3	5.1	.54					16					3.4	4.6	3.2	2.9	3.5	.85					
5	4.9	.85	4.5	0	25	56	18	3.0	1.2	2.0	.55	4.2	4.6	3.7	3.2	3.9	.76	16 x 6	0			
7	4.1	.84					15					4.2	3.5	3.3	3.3	3.6	.79					
7.6	7.6	1.00	3.5	0	18	54	15	2.9	4.4	5.1	.86	7.1	7.1	4.9	7.1	6.5	1.00	16 x 5	0			
5.5	6.2	1.03					16					5.2	5.7	4.3	4.5	5.0	.88					
3.0	6.3	.91	4.5	0	22	75	16	2.7	3.9	4.6	.83	5.3	5.3	4.8	4.8	5.0	.91	16 x 6	0			
4.8	5.1	.94					18					4.3	4.7	4.0	4.0	4.4	.85					
5.0	5.3	.85	3.7	-----	18.7	41	16	4.6	3.1	4.9	.63	5.8	5.4	4.9	4.7	5.3	.81	15.4 x 5.3				
2.8	6.6	.28	18	2L														20 x 7	3 L			
3.6	6.0	.42	18	2L																		
2.7	4.5	.42	8																			
2.4	3.5	.45	8																			
---	-----	-----	4.5	-----	16	61	-----	3.8	4.6	5.3	.87	-----	-----	-----	-----	-----	-----	27 x 9	3 L			
3.4	4.9	.51	5.5	2½L [1]	20	51	33	4.1	4.5	5.6	.80	7.8	7.6	7.5	7.2	7.6	.92	22 x 6	2 L (1 NEM)			
2.7	4.2	.43					31					7.1	7.1	6.8	5.4	6.6	.76					
2.9	4.9	.42	10.3	-----	18	56	32	4.0	4.6	5.5	.84	7.5	7.4	7.3	6.3	7.1	.84	23 x 8				
1.8	5.1	.18	6.0	4L	20	29	15	6.8	3.5	4.7	.75	6.8	4.7	3.9	1.6	4.3	.24	18 x 5	2½ L			
1.7	4.4	.21					15					5.7	3.9	2.9	1.3	3.5	.23					
6.9	8.9	-----	Moderately enlarged.	2½L	16	67	25	4.1	5.2	6.6	.79	-----	11.4	5.2	4.6	7.1	-----	Greatly enlarged.	3 L			
6.5	7.9	-----					27					-----	9.7	3.7	4.2	4.4						

| | | |

	Liver																	
		Wet weight		Aerobic				Anaerobic										
								$Q^{N_2}{}_A$										
								Hour										
and of tion	Dry weight (initial)	Aerobic	Anaerobic	$Q^{O_2}{}_A$	Q_{CO_2}	Q_{O_2}	R. Q.	1st	2d	3d	4th	Average	Ratio 4th/1st hour	Liver size	Degree and type [1] of infiltration
	Pct.	*Mg.*	*Mg.*											*Mm.*	
EM)	21.3	126	40	0.42	0.50	1.4	.....	.....	0.43	0.43	0.14	0.25	.....	N [2]	0.
		126	40	.51	.....	1.2	.....	(0.14)	.43	.29	.14	.25	.....		
EM)	[4] 19.4	127	43	.33	.93	1.6	0.58	.1	.1	.16	.81	.29	.....	N	0.
		126	44	.44	1.1	1.8	.61	0	0	.17	.66	.21	.....		
	20.3	126	42	.42	.84	1.5	.60	(.08)	.24	.28	(.44)	.25	.....	N	
														N	0.
														N	0.
														N	
														N	
EM)														N	
														N	
														N	
														N	
														N	
	18	103	43	2.2	4.0	5.3	.76	10.1	9.3	4.6	3.7	6.9	0.37	Enormous	4 L.
		103	41	2.2	4.1	5.0	.82	7.8	7.8	7.6	3.6	6.7	.46		
	18	128	.....	1.8	1.5	2.2	.68	.....	.....	.....	.....	.....	.....	Slightly enlarged.	1½ L.
		127	.....	1.6	1.6	2.2	.73	.....	.....	.....	.....	.....	.....		
NEM)	19	102	30	2.3	4.7	5.5	.86	4.8	5.0	4.8	3.0	4.4	.62	Slightly enlarged.	2½ L.
		100	33	1.6	5.8	7.1	.82	6.3	6.3	5.0	5.0	5.7	.79	Moderately enlarged.	
	18	110	37	1.9	3.6	4.5	.78	7.2	7.1	5.5	3.8	5.9	.56		
	20	104	30	1.1	1.7	2.6	.65	3.4	2.5	1.3	1.1	2.1	.32	Very slightly enlarged.	2¾ L.
		102	31	1.7	2.0	2.4	.84	3.6	2.1	1.5	1.1	2.1	.31	Probably enlarged.	4 L.
	(18)	124	34	1.9	4.9	6.1	.80	.....	8.4	3.4	3.0	4.7	.....	Very slightly enlarged.	
		125	34	2.0	4.4	5.9	.75	.....	8.9	2.9	2.9	4.7	.....		
	19	114	32	1.7	3.2	4.2	.76	(3.5)	5.5	2.3	2.0	3.4	(.32)		
	17	102	42	3.4	1.9	3.0	.63	.....	5.9	3.8	3.6	4.4	.....	Slightly enlarged.	
		100	42	3.2	1.8	2.5	.72	.....	4.9	3.9	3.1	4.0	.....	.....	3 [7]
		127	33	3.4	3.9	6.0	.65	12.1	11.8	9.6	10.4	11.0	.86	Not enlarged	3 [7]

164m, 165f, 166m, 167f	Oct. 7	(16)	–	–	.....	27.8	75	20	2.3	2.5	2.8	.89	3.5	3.0	3.0	3.0	3.1	.86	2	0	20.0	
								20					3.2	3.0	3.0	3.0	3.0	.94				
269m, 270m, 271m, 272m, 273m, 274m	Dec. 11	(16.5)	–	–	.....	(35.5)	75	32	2.2	3.3	4.7	.78	7.8	7.6	6.7	5.9	7.0	.76	2	0	18.0	
								31					7.1	6.1	5.5	3.1	5.4	.44				
260m, 261m, 262f, 263f, 264m	Dec. 5	(23.5)	–	–	.....	[4] 17.7	65	23	1.4	3.6	4.6	.78	7.2	9.9	6.9	6.9	7.7	.96	2	0	[4] 19.3	
								25					6.7	5.4	5.0	5.7	5.7	.85				
Average	.....	(17.5)	.....	.....	.....	25.9	73	23	2.1	2.9	3.7	.82	5.3	4.9	4.3	3.9	4.6	.74	2	.....	18.7	
Painted, negative (preleukemic): 124m, 125m, 126f	Aug. 16	7	–	–	[5]	20	76	43	.....	3.1	3.9	.80	5.4	6.1	6.3	5.2	5.7	.96	3.5	0	.....	
								44					4.9	5.3	5.4	4.3	5.0	.88				
129f, 130m, 131f	Aug. 23	8	–	–	[5]	15	76	25	2.9	4.0	4.8	.84	7.5	6.6	6.3	5.9	6.6	.79	3.5	0	.....	
								25					7.3	5.6	5.3	4.9	5.8	.67				
134f, 135f, 136m	Aug. 30	9	–	–	[5]	15	76	18	3.0	2.4	3.7	.66	6.0	5.0	5.3	4.5	5.2	.75	3.5	0	15	
								18					4.5	5.3	5.1	4.5	4.8	1.00				
137m, 138f, 139m	Sept. 6	10	–	–	[5]	16	63	16	2.9	2.1	3.1	.69	5.0	4.8	4.6	.....	4.8	.....	3.5	0	16	
								15					4.6	4.6	4.4	.....	4.5	.....				
144f, 145m, 146m	Sept. 13	11	–	–	[5]–	16	60	15	3.7	2.1	2.8	.77	5.3	4.2	4.0	4.8	4.6	.91	3.5	0	18	
								15					3.8	3.5	3.5	3.3	3.5	.87				
149f, 150m, 151f	Sept. 20	12	[6]±	[6]±	[6]±	17	75	16	3.2	1.5	1.9	.80	7.5	7.5	6.1	5.6	6.7	.75	3.5	0	17	
								16					6.1	6.4	4.7	3.3	5.1	.54				
152m, 153f, 154m	Sept. 27	13	–	–	[5]–	17	103	17	2.8	2.3	2.7	.83	5.3	5.0	4.7	4.5	4.9	.85	4.5	0	25	
								18					4.4	4.7	3.7	3.7	4.1	.84				
160m, 161m, 162f	Oct. 4	14	–	–	[5]–	15	61	17	2.4	4.2	4.4	.94	7.6	7.8	7.6	7.6	7.6	1.00	3.5	0	18	
								19					6.3	6.3	5.9	6.5	6.2	1.03				
169f, 170f, 171m	Oct. 11	15	–	–	[5]–	18	75	16	.....	.....	3.7	.84	6.6	6.8	5.8	6.0	6.3	.91	4.5	0	22	
													5.1	5.9	4.6	4.8	5.1	.94				
Average	.....	11	.....	.....	.....	16.6	73	20	3.0	2.7	3.4	.80	5.5	5.6	5.2	5.0	5.3	.85	3.7	.....	18.7	
Painted, leukemic: 212f	Oct. 18	16	+	+	+	[16	103	20	2.9	3.5	4.9	.70	9.9	8.1	5.5	2.8	6.6	.28	18	2L	.....	
						[15	103	17	2.7	3.1	4.5	.69	8.6	7.3	4.7	3.6	6.0	.42	18	2L	.....	
							102	20	2.8	4.0	4.7	.84	6.5	5.4	3.2	2.7	4.5	.42	8	.....	.....	
							103	20	2.7	3.3	3.7	.89	5.3	3.7	2.6	2.4	3.5	.45	8	.....	.....	
222m	Oct. 23	17	+	+	+	15	45	.....	4.0	3.2	4.8	.74	.....	.....	.....	.....	.....	.....	4.5	.....	16	
246m	Nov. 20	21	+	+	+	21	76	16	2.6	5.1	6.0	.86	6.7	5.5	3.9	3.4	4.9	.51	5.5	2½L [7]	20	
								15					6.3	3.6	4.3	2.7	4.2	.43				
Average	.....	18	.....	.....	.....	16.8	89	18	3.0	3.7	4.8	.79	7.2	5.6	4.0	2.9	4.9	.42	10.3	.....	18	
Transmitted (unpainted): 240f, 241f (2d transfer from 150; inoculated Oct. 11, 1940).	Nov. 4	(3)	+	+	+	21	76	17	3.7	4.3	4.0	(1.06)	9.8	5.4	3.5	1.8	5.1	.18	6.0	4L	20	
								17					8.2	4.5	3.0	1.7	4.4	.21				
265m (2d transfer from 212; inoculated Nov. 26, 1940).	Dec. 7	(1½)	+	+	+	16.7	76	30	2.3	4.4	5.9	.75	.....	12.6	7.1	6.9	8.9	.....	Moderately enlarged.	2½L	16	
								28						11.0	6.3	6.5	7.9	.....				
Average	.....	(2)	.....	.....	.....	18.8	76	23	3.0	4.4	5.0	(.91)	(9.0)	8.4	5.0	4.2	6.6	(.20)	6.0	.....	18	
Painted, leukemoid (nontransmissible): 245m	Nov. 15	20	–	–	–	11.0	42	15	3.6	3.2	3.8	.85	2.5	2.5	2.8	3.7	2.9	(1.48)	4	—	29	
247m	Dec. 3	23	–	–	–	16.7	75	23	4.5	6.3	6.6	.96	12.4	11.3	10.6	11.9	11.5	.96	6	—[7 8]	24	
								22					11.7	12.3	10.0	10.0	11.0	.85				
Average	.....	22	.....	.....	.....	13.9	59	20	4.0	4.8	5.2	.91	8.9	8.7	7.8	8.5	8.5	(1.10)	5	.....	27	
Spontaneous (unpainted): Ak/C3H 131f [11]	Mar. 25	[11] (34)	+	+	.....	14.7	102	44	3.0	4.3	6.0	.72	12.1	9.7	8.2	6.5	9.1	.54	Greatly enlarged.	3L	15	
C3H/Ak 74f [11]	Apr. 19	[11] (40)	+	+	.....	16.0	72	30	5.1	5.0	6.8	.74	10.4	10.1	6.8	6.6	8.5	.63	.....	2 L [13]	.....	
								30					10.1	9.5	6.3	6.2	8.0	.61				
Average	.....	.....	.....	.....	.....	15.4	87	35	4.1	4.7	6.4	.73	10.9	9.8	7.1	6.4	8.5	.59	.....	.....	15	

[1] Time after painting at 4–5 weeks old; parenthetical values are for unpainted animals of equivalent age calculated from 4–5 (not 0) weeks old, or for transmitted values referring to period after inoculat
[2] See footnotes 3 and 5, table 1.
[3] Normal.
[4] Final dry weight percentages (on material taken from aerobic vessels) are for lymph nodes, spleen, and liver of Rf 260–264, respectively: 13.2, 9.5, and 10.9 as compared with initial dry weight perc of 17.7, 19.3, and 19.4, or final/initial dry weight ratios of 0.75, 0.49, and 0.56; for Rf 265, 9.0, 5.2, and 7.9 as compared with 16.7, 16.0, and 18.0, or ratios of 0.53, 0.33, and 0.44.
[5] Nos. 125, 131, 134, 139, 146, 151, 154, 156–162, 164–167, 171, 240, 241 not tested for inoculation.
[6] No. 150, 3L. The proportion of malignant Rf 150 material admixed with the preleukemic Rf 149 and 151 material had no evident effect upon the observed metabolic values.
[7] Some nonmalignant reticulo-endothelial cells, eosinophiles.

eatly enlarged.	[7 8] (1)	17	102	42	3.4	1.9	3.0	.63		5.9	3.8	3.6	4.4		Slightly enlarged.															
			100	42	3.2	1.8	2.5	.72		4.9	3.9	3.1	4.0			3 [9]														
24	[7 8 10] (1)		127	33	3.4	3.9	6.0	.65	12.1	11.8	9.6	10.4	11.0	.86	Not enlarged	3.7														
20.0	[75	20	3.6	2.3	3.6	.64	6.2	5.0	4.9	4.9	4.8	.83																		
		21					5.3	5.0	4.4	4.4	4.8	.83																		
18.0	[	30					8.1	6.4	5.8	5.4	6.4	.67	15 x 5	0 (1 NEM)	21.3	[126	40	0.42	0.50	1.4										
		32					6.6	5.7	5.3	4.0	5.4	.61				126	40	.51		1.2		(0.14								
[4] 19.3	[64	22	2.4	3.9	5.3	.74	7.4	7.8	6.9	6.7	7.2	.90	15 x 5	0 (1 NEM)	[4] 19.4	[127	43	.33	.93	1.6	0.58	.1								
		21					6.1	7.6	6.9	6.5	6.8	1.07				126	44	.44	1.1	1.8	.61	0								
18.7	72	22	3.2	2.9	4.1	.71	5.8	5.6	4.7	4.4	5.1	.76	14.7 x 4.7		20.3	126	42	.42	.84	1.5	.60	(.08								
													16 x 6	0																
													16 x 5	0																
15	[28	15	5.2	2.0	4.6	.44	7.7	6.0	5.3	5.5	6.1	.71	15 x 5	0																
		17					6.5	5.8	5.5	5.2	5.8	.80																		
16	[25	15	6.6	3.2	6.4	.50	7.4	7.1	7.4		7.3		14 x 5	0																
		15					4.7	4.4	4.7		4.6																			
18	[29	15	4.3	4.9	6.2	.79	9.1	6.2	7.0	6.8	7.3	.75	15 x 5	0 (3 NEM)																
		15					9.1	5.9	7.6	5.6	7.1	.62																		
17	18	14	7.2	2.2	5.4	.41	4.7	5.1	4.0	3.4	4.3	.72	15 x 5	0																
		16					3.4	4.6	3.2	2.9	3.5	.85																		
25	[56	18	3.0	1.2	2.0	.55	4.2	4.6	3.7	3.2	3.9	.76	16 x 6	0																
		15					4.2	3.5	3.3	3.3	3.6	.79																		
18	[54	15	2.9	4.4	5.1	.86	7.1	7.1	4.9	7.1	6.5	1.00	16 x 5	0																
		16					5.2	5.7	4.3	4.6	5.0	.88																		
22	[75	16	2.7	3.9	4.6	.83	5.3	5.3	4.6	4.8	5.0	.91	16 x 6	0																
		18					4.3	4.7	4.0	4.0	4.4	.85																		
18.7	41	16	4.6	3.1	4.9	.63	5.8	5.4	4.9	4.7	5.3	.81	15.4 x 5.3																	
													20 x 7	3 L	18	[103	43	2.2	4.0	5.3	.76								
																103	41	2.2	4.1	5.0	.82								
16	61		3.8	4.6	5.3	.87							27 x 9	3 L	18	[128		1.8	1.5	2.2	.68								
																127		1.6	1.6	2.2	.73								
20	51	33	4.1	4.5	5.6	.80	7.8	7.6	7.8	7.2	7.6	.92	22 x 8	2 L (1 NEM)	19	[102	30	2.3	4.7	5.5	.86								
		31					7.1	7.1	6.8	5.4	6.6	.76				100	33	1.6	5.8	7.1	.82								
18	56	32	4.0	4.6	5.5	.84	7.5	7.4	7.3	6.3	7.1	.84	23 x 8		18	110	37	1.9	3.6	4.5	.78									
20	[29	15	6.8	3.5	4.7	.75	6.8	4.7	3.9	1.6	4.3	.24	18 x 5	2½ L	20	[104	30	1.1	1.7	2.6	.65									
		15					5.7	3.9	2.9	1.3	3.5	.23				102	31	1.7	2.0	2.4	.84									
16	[67	25	4.1	5.2	6.6	.79		11.4	5.2	4.6	7.1		Greatly enlarged.	3 L	(18)	[124	34	1.9	4.9	6.1	.80									
		27						9.7	3.7	4.2	4.4					125	34	2.0	4.4	5.9	.75									
18	48	21	5.5	4.4	5.7	.77	(6.3)	7.4	3.9	2.9	4.8	(.23)	18 x 15		19	114	32	1.7	3.2	4.2	.76									
29	[56	40	3.4	4.6	5.9	.78	5.9	5.5	5.1	5.1	5 4	.86	Greatly enlarged.	[7 8] (1)	17	[102	42	3.4	1.9	3.0	.63									
		40					5.2	4.5	3.9	3.9	4.4	.75				100	42	3.2	1.8	2.5	.72									
24	[100	31	3.9	4.2	5.6	.75	10.1	8.9	8.4	8.0	8.9	.79	8 x 24	[7 8 10] (1)	16	[127	33	3.4	3.9	6.0	.65									
		31					9.7	8.5	8.4	7.7	8.6	.79				127	33	3.3	3.6	4.7	.77									
27	78	35	3.7	4.4	5.8	.77	7.7	6.8	6.4	6.2	6.8	.80	8 x 24		17	114	38	3.3	2.8	4.0	.69									
15	101	42	3.8	6.6	8.9	.74	15.3	12.1	10.1	8.3	11.4	.54	Greatly enlarged	3 L (1 NEM)	23	[102	40	.93	3.9	5.5	.72									
																103	41	.81	3.1	4.5	.69									
	[101	30	4.2	6.3	8.0	.79	11.3	12.9	8.5	7.4	10.1	.63		2 A [11]																
	[102	30	4.5	5.9	7.9	.75	10.0	10.2	7.0	6.7	8.5	.67																		
	[101		4.1	4.9	6.6	.74																								
15	101	34	4.1	5.9	7.8	.75	12.3	11.7	8.5	7.5	10.0	.61			23	103	41	.87	3.5	5.0	.71									

After inoculation.

weight percentage values

[8] Some erythoblasts.
[9] Some polymorphonuclear leukocytes.
[10] Some giant cells and megakaryocytes.
[11] Animals were unpainted, and the age given in the column "Weeks after painting" refers to the ages at the time the animals were killed
of hybrids are fully susceptible to Rf and Ak and Rf/Ak leukemias.
[12] Most infiltrated cells in the lymph nodes were lymphoid, with large mononuclear and reticulo-endothelial cells; the splenic infiltration w
karyocytes, and erythroblasts in addition.

4.5 4.4	4.8 4.8	.87 .83	15 x 4	0																
5.4 4.0	6.4 5.4	.67 .61	15 x 5	0 (1 NEM)	21.3	126 126	40 40	0.42 .51	0.50	1.4 1.2		(0.14)	0.43 .43	0.43 .29	0.14 .14	0.25 .25		N [3]	0.
6.7 6.5	7.2 6.8	.90 1.07	15 x 5	0 (1 NEM)	[4] 19.4	127 126	43 44	.33 .44	.93 1.1	1.6 1.8	0.58 .61	.1 0	.1 0	.16 .17	.81 .66	.29 .21		N	0.
4.4	5.1	.76	14.7 x 4.7		20.3	126	42	.42	.84	1.5	.60	(.08)	.24	.28	(.44)	.25		N	
			16 x 6	0														N	0.
			16 x 5	0														N	0.
5.5 5.2	6.1 5.8	.71 .80	15 x 5	0														N	
	7.3 4.6		14 x 5	0														N	
6.8 5.6	7.3 7.1	.75 .62	15 x 5	0 (3 NEM)														N	
3.4 2.9	4.3 3.5	.72 .85	15 x 5	0														N	
3.2 3.3	3.9 3.6	.76 .79	16 x 6	0														N	
7.1 4.6	6.5 5.0	1.00 .88	16 x 5	0														N	
4.8 4.0	5.0 4.4	.91 .85	16 x 6	0														N	
4.7	5.3	.81	15.4 x 5.3																
			20 x 7	3 L	18	103 103	43 41	2.2 2.2	4.0 4.1	5.3 5.0	.76 .82	10.1 7.8	9.3 7.8	4.6 7.6	3.7 3.6	6.9 6.7	0.37 .46	Enormous	4 L.
			27 x 9	3 L	18	128 127		1.8 1.6	1.5 1.6	2.2 2.2	.68 .73							Slightly enlarged.	1½ L.
7.2 5.4	7.6 6.6	.92 .76	22 x 8	2 L (1 NEM)	19	102 100	30 33	2.3 1.6	4.7 5.8	5.5 7.1	.86 .82	4.8 6.3	5.0 6.3	4.8 5.0	3.0 5.0	4.4 5.7	.62 .79	Slightly enlarged. Moderately enlarged.	2½ L.
6.3	7.1	.84	23 x 8		18	110	37	1.9	3.6	4.5	.78	7.2	7.1	5.5	3.8	5.9	.56		
1.6 1.3	4.3 3.5	.24 .23	18 x 5	2½ L	20	104 102	30 31	1.1 1.7	1.7 2.0	2.6 2.4	.65 .84	3.4 3.6	2.5 2.1	1.3 1.5	1.1 1.1	2.1 2.1	.32 .31	Very slightly enlarged. Probably enlarged.	2¾ L.
4.6 4.2	7.1 4.4		Greatly enlarged	3 L	(18)	124 125	34 34	1.9 2.0	4.9 4.4	6.1 5.9	.80 .75		8.4 8.9	3.4 2.9	3.0 2.9	4.7 4.7		Very slightly enlarged.	4 L.
2.9	4.8	(.23)	18 x 15		19	114	32	1.7	3.2	4.2	.76	(3.5)	5.5	2.3	2.0	3.4	(.32)		
5.1 3.9	5.4 4.4	.86 .75	Greatly enlarged	[7] [8] (1)	17	102 100	42 42	3.4 3.2	1.9 1.8	3.0 2.5	.63 .72		5.9 4.9	3.8 3.9	3.6 3.1	4.4 4.0		Slightly enlarged.	3 [7] [9]
8.0 7.7	8.9 8.6	.79 .79	8 x 24	[7] [9] [10] (1)	16	127 127	33 33	3.4 3.3	3.9 3.6	6.0 4.7	.65 .77	12.1 10.6	11.8 9.4	9.6 9.9	10.4 8.5	11.0 9.5	.86 .80	Not enlarged Very slightly enlarged.	3.7
6.2	6.8	.80	8 x 24		17	114	38	3.3	2.8	4.0	.69	11.4	8.0	6.7	6.4	7.2	.83		
8.3	11.4	.54	Greatly enlarged	3 L (1 NEM)	23	102 103	40 41	.93 .81	3.9 3.1	5.5 4.5	.72 .69	6.8 5.8	5.2 4.6	(4.0) 3.5	(3.5) 2.9	4.9 4.2	.51 .50		3 L (fatty degeneration).
7.4 6.7	10.1 8.5	.63 .67		2 A [12]															
7.5	10.0	.61			23	103	41	.87	3.5	5.0	.71	6.3	4.9	3.8	3.2	4.6	.51		

s.
es.
ge given in the column "Weeks after painting" refers to the ages at the time the animals were killed for metabolic studies of spontaneous leukemias developed. These types
d Ak and Rf/Ak leukemias.
nodes were lymphoid, with large mononuclear and reticulo-endothelial cells; the splenic infiltration was composed of atypical cells), with polymorphonuclear leukocytes, mega-
on.

when one considers the first or second hour $Q^{N_2}{}_A$ values rather than average $Q^{N_2}{}_A$ values. The fourth to first hour $Q^{N_2}{}_A$ ratios (as in table 1) are lowest in the leukemic group, so that the first or second hour leukemic values tend to be higher, but the third and fourth hour values lower. As already indicated, cell fragility is probably involved here. There is a great increase in organ size as one proceeds through control and preleukemic to leukemic groups, with, however, no clear-cut influence of this factor on metabolism, even though the great size increase is undoubtedly connected with the leukemic development.

In the leukemic Rf livers there are even more marked relative increases in anaerobic glycolysis than in the leukemic Rf/Ak livers, and indeed the absolute values of the Rf leukemic livers appear (particularly for the first 2 hours) to be definitely greater than those of the leukemic Rf/Ak livers, although the slightly greater infiltration and longer period of painting in the case of the Rf livers may account for this. Average $Q^{N_2}{}_A$ values well over 5 (up to 7) were obtained; admittedly only two cases were observed, and the two transmitted leukemic liver values were not so high as the primary leukemic values. It is an interesting reflection that in the two mouse stocks in which leukemia was induced, it was not the Rf/Ak stock with the higher eventual spontaneous incidence, but rather the Rf stock with the lower spontaneous incidence, that yielded the higher absolute anaerobic glycolysis values in liver. This may be related in some way to the fact that longer periods of painting were involved in the higher glycolysis, lower spontaneous-incidence Rf stock. However, the shorter periods involved with the Rf/Ak stock, studied not simultaneously but following the Rf series, might be due simply to improved technique. As an additional factor, the degrees of infiltration observed in the particular livers taken for metabolism very probably represent variability in time elapsing between the onset of leukemia and metabolic analysis. In the Rf/Ak series, with the lower metabolic values, this time happened to be seldom more than a few days to a week, in contrast with that in the Rf series where somewhat longer periods were usually involved, and in still greater contrast with the two spontaneous cases observed where the time was certainly far greater, and the metabolic values unquestionably higher. Further work on the effect of length of leukemic period, as a factor distinct from degree of infiltration, is obviously suggested.

Data for preleukemic Rf livers are not available since their possible importance was not discovered in this experimental series (actually carried out before the Rf/Ak series) until leukemic livers were obtained, by which time preleukemic sibling mice were no longer available. It was the enormous size of the Rf 212 liver that suggested the taking of observations on liver in connection with the painted mice reported in table 1. As indicated in the discussion of table 1 and confirmed by the data of table 2, observations on liver are evidently of considerably greater potential interest than those on lymph nodes or spleens, especially in connection with leukemic and preleukemic changes.

The two (unpainted) Rf mice with transmitted leukemias gave rather widely differing anaerobic glycolysis values in each of the three organs examined. Admittedly, with this inconsistency, it cannot be said that the values for the transmitted leukemias are definitely different from those of the primary leukemias, except possibly that they are smaller in the case of liver. Likewise the two cases of leukemoid infiltrations gave widely differing values in each of the three organs, so that again it

cannot be said that leukemoid values are, statistically considered, different from the primary or transmitted leukemias. In the case of the Rf 245 lymph nodes the $Q^{N_2}{}_A$ values would be considerably higher than they are, and nearer those of Rf 247, if the measured, very low, dry-weight value of 11 percent were employed rather than the assumed 18-percent value actually used.

In our judgment the Rf 247 values are to be preferred over the Rf 245 values, about which there was some technical uncertainty. The Rf 247 values are preferable both because of the greater tissue weight employed, and in particular because of the available chemical Q_{LA} values which confirm the Q_A values. Our feeling is that a statistically adequate study of many leukemoid cases might well indicate average glycolysis values higher than the value for leukemic, preleukemic, or control mice; this is certainly true for one particular leukemoid instance (Rf 247), where there is no reason to question the very high experimental values obtained. On the other hand, as shown in table 4, Hall (*12*) obtained metabolic values in Rf mice for (leukemoid) nonmalignant myeloid infiltration in lymph nodes and spleen that were on the whole a little smaller than the Rf leukemoid values reported in table 2. The mice employed by Hall, however, were considerable older.

Whatever difficulties there may be in establishing any metabolic differences between normal or leukemoid lymph nodes and spleens of Rf mice, we believe that such uncertainty does not exist in regard to the two cases of spontaneous leukemias studied. In the latter we have a confirmation and quantitative replication of the work of previous investigators, who have observed definite increases of some 100 percent or more in lymph nodes of mice with spontaneous lymphoid leukemia as compared with normal nodes. The certainty of difference in the case of the spontaneous leukemias observed in confirmation of the work of others, enhances our conclusion regarding the lack of any notable difference in the case of the lymph nodes and spleen of the induced Rf and Rf/Ak leukemias as compared with the controls.

Aerobic Metabolism

The average respiratory quotients of all tissues examined were well below unity, in general decreasing slightly in the order of lymph nodes, spleen, liver. An R. Q. well below unity is characteristic of both adult normal tissue and malignant tissue, in contrast with almost all growing normal tissue, where the R. Q. often attains unity. The average R. Q. values of the leukemoid nodes appeared to be 0.1 nearer unity than the leukemic or normal nodes, but this trend was not noted with respect to spleen or liver. R. Q. values, especially in liver, varied considerably, but as shown in table 2 they were all well below unity, and in fact many were below 0.75 or even 0.7.

We have hesitated to conclude that there is any statistically significant difference (greater than 20 percent) in anaerobic metabolism of lymph node or spleen, between any of the control, preleukemic. leukemic, or leukemoid groups (save the two spontaneous leukemias). Nevertheless, it is believed necessary to conclude from the data of table 2 that the leukemic and leukemoid groups possess an average oxidative (Q_{O_2} and Q_{CO_2}) metabolism some 25 to 75 percent higher than the normal and preleukemic groups in the Rf strain measured. Such a respiratory difference between leukemic (or leukemoid) and nonleukemic organs has never been reported before, except by Victor and Wintersteiner (*10*) for their lines A and M-spleen-D on mouse strain C58, with respect to

lymph nodes. In the case of the aerobic liver values reported in table 2 the relative difference between leukemic (or leukemoid) and control groups is very striking, being upwards of several hundred percent. This is true if the data on the control sets of mice can be relied upon literally. The particular values obtained are certainly smaller than usual for normal mouse liver. In view of the wide difference in values for control and leukemic livers, it is unfortunate that there are no aerobic data available for preleukemic livers, since here a wide range over which to observe possible preleukemic changes in metabolism would be available. This is true not only for respiration but also for aerobic glycolysis.

The relative and absolute increases of $Q^{O_2}{}_A$ of the leukemic livers are not so great as the anaerobic $Q^{N_2}{}_A$ increases. This is almost invariably the case in all tissues because of the operation of the Pasteur effect, which results in a definitely smaller aerobic than anaerobic glycolysis. Also, it is likely that some of the lactic acid produced by the infiltrating cells will be oxidized by the liver cells under aerobic conditions. It should be remembered that a liver infiltrated with a considerable proportion of leukemic cells is a mixture of two metabolically different types of tissue, the joint action of which may well be expected to be different from the sum of the actions of the component tissues analyzed separately. This is particularly true in the presence of oxygen, where an enhanced or multiple Pasteur effect might be expected with mixed tissue.

In the case of lymph nodes and spleen reported in table 2, there is definite experimental indication that the aerobic glycolysis is greater by 50 to 100 percent, not only in the leukemic (or leukemoid) but also preleukemic mice as compared with the unpainted controls. The trend would be even more pronounced in the case of lymph nodes if all $Q^{O_2}{}_A$ values were calculated upon a basis of the initial-dry-weight percentages (rather than the arbitrarily chosen 18-percent dry weight), since with three of the four control values the measured dry-weight values were well above 18 percent (about 25, 50, and 100 percent above, respectively). However, it can be said, even on the measured dry-weight basis (and practically so on an 18-percent dry-weight basis), that no single $Q^{O_2}{}_A$ lymph node value in the control group was as high as the lowest value in any of the preleukemic, leukemic, or leukemoid groups. Almost the same may be said regarding the spleen $Q^{O_2}{}_A$ values.

In attempting to interpret the increase in the $Q^{O_2}{}_A$ in the experimental over the control group, it is possible to fall back on the time-worn explanation that some incidental artifact or differential damage resulting from difference in organ size is involved. This is, however, as in the case of most such previous claims, *ad hoc*, and without independent substantiation. As another *ad hoc* interpretation, it is just as likely that there is some mutual or synergistic effect obtained between the organs and the infiltrations, acting, however, in these metabolically more related mixed tissues, to produce the increased $Q^{O_2}{}_A$ values, rather than decrease them as in the case of the infiltrated liver mentioned above, where the mixed tissues are metabolically more different. This, however, would not cover the preleukemic nodes or spleen, where there is no leukemic infiltration. Here a definite experimental explanation or interpretation might well be based upon the rather extensive hyperplasia noted in almost all preleukemic spleens and lymph nodes. We are inclined to believe that, in the preleukemic

group at any rate, the hyperplasia might well account for the observed increased aerobic glycolysis. This brings up the question whether the hyperplasia is to be regarded as a necessary or an incidental factor in the development of eventual leukemia; in other words, whether the preleukemic hyperplasia represents a definite preleukemic state as defined in the introduction.

The aerobic metabolic values of the transmitted leukemias are on the whole indistinguishable from the leukemic values in all three organs examined. The respiration and aerobic glycolysis values of the spontaneous nodes examined are the same as, or a little greater than, those of the induced or transmitted lymphoid leukemias of table 2.

The aerobic metabolic values in the two cases of nontransmissible leukemoid infiltrations studied are, as in the case of the anaerobic values, markedly different, in both lymph nodes and in liver (though not in spleen). The difference between Rf 245 and 247 in the case of the lymph nodes is somewhat decreased, if the initial measured rather than the assumed dry weight is employed. In any event they more nearly resemble the leukemic rather than the control group, and in our opinion their average metabolism is, if anything, higher than the leukemic. This is of especial interest in view of the apparent nonmalignancy of these nontransmissible leukemoid cases, for which, together with figures 1 and 2, the following detailed, supporting histologic descriptions are given. For the present these do not appear to throw any evident light on the metabolic differences between the two mice, apart from the facts that Rf 247 had larger nodes (greater hyperplasia), relatively more erythrogenic cells, far fewer eosinophiles, and glandlike inclusions in the spleen (none in Rf 245 spleen).

Rf 245.—Mouse Rf 245 was 6 months old and profoundly anemic and dying when killed for metabolism studies 20 weeks after painting had begun. There were several papillomas in the skin; the base of two large papillomas measured 10 to 15 mm. across and appeared ill-defined. The superficial lymph nodes were slightly enlarged and measured approximately 4 mm. in greatest diameter. At autopsy the deep lymph nodes were not enlarged.

The spleen was pale, gray-pink, measuring 24 by 8 mm. in the two greatest diameters. The liver was brown-red, pale, and of normal size. There were approximately 25 minute tumors (adenomas) in the lung. Microscopic examination of the liver showed advanced leukocytic infiltration (fig. 2). Most leukocytes were mature, but there were young polymorphonuclear leukocytes and many eosinophiles. There were also a few lymphocytes, leukocytes, and monocytes, and a variable number of fibroblastlike cells. Fibrosis was marked in some places, negligible in others. In addition to these infiltrations which were most conspicuous in the portal area, there were in the liver scattered small foci of myelopoiesis and scattered megakaryocytes. In the foci of myelopoiesis, the cells were myelocytes in various stages of maturity. Mitotic figures were numerous. A section of the spleen showed replacement of the organ by cells similar to those seen in the liver. The lymphoid follicles were small or absent altogether. The overwhelming number of cells were myelogenic. Eosinophiles and mature forms were abundant, but relatively less numerous than in the liver. Earlier forms of myelocytes and mitotic figures, on the contrary, were more numerous in the spleen than in the liver. Erythroblasts were few, megakaryocytes numerous. Fibrosis was inconspicuous. The lymph node was not examined microscopically. The bone marrow showed hyperplasia of granulocytes with an unusually large number of eosinophiles and many mature forms.

Rf 247.—The gross and microscopic changes in mouse Rf 247 were similar to those in Rf 245. The animal was very pale. The superficial lymph nodes measured from 5 to 7 mm. in greatest diameter, and the spleen measured 24 by 8 mm. in the two greatest diameters. There were numerous lung tumors, cutaneous papillomas, and a few carcinomas arising in papillomas. The microscopic changes in the liver were essentially the same as in Rf 245. In the spleen, however, areas of fibrosis were more conspicuous, and there were, in addition to cells like those seen in Rf 245, numerous multinucleated giant cells (fig. 3), which occurred in the areas of fibrosis.

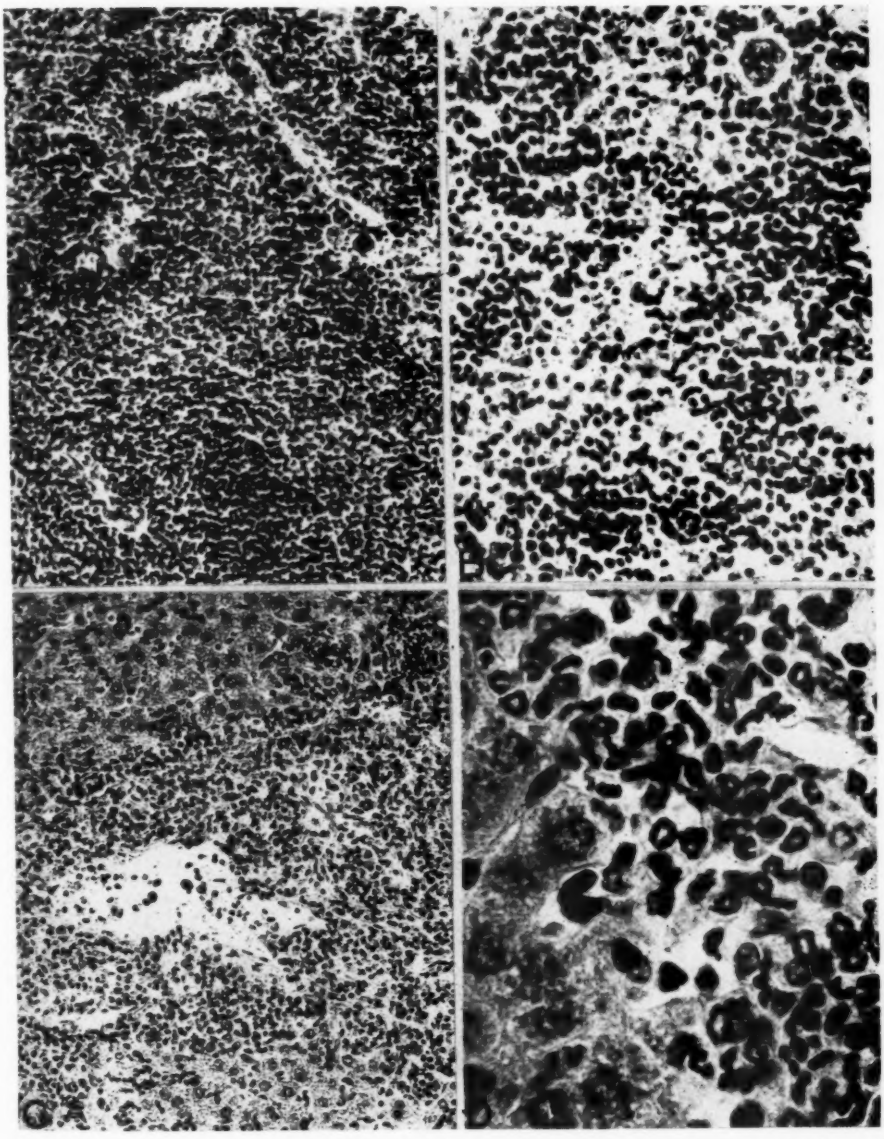

FIGURE 2.—*A*, Spleen of mouse Rf 135 that had been painted with methylcholanthrene for 9 weeks, showing extramedullary myelopoiesis, mainly erythropoiesis. A large lymphoid follicle is seen close to the lower margin. × 75; *B*, Higher magnification of *A* showing mainly erythrogenic centers with few myeloid cells between them. × 200; *C* and *D*, Liver of painted mouse Rf 245 showing extensive myeloid infiltration in the portal area, mostly with mature myeloid cells. × 120 and 900, respectively. All sections stained with haematoxylin and eosin.

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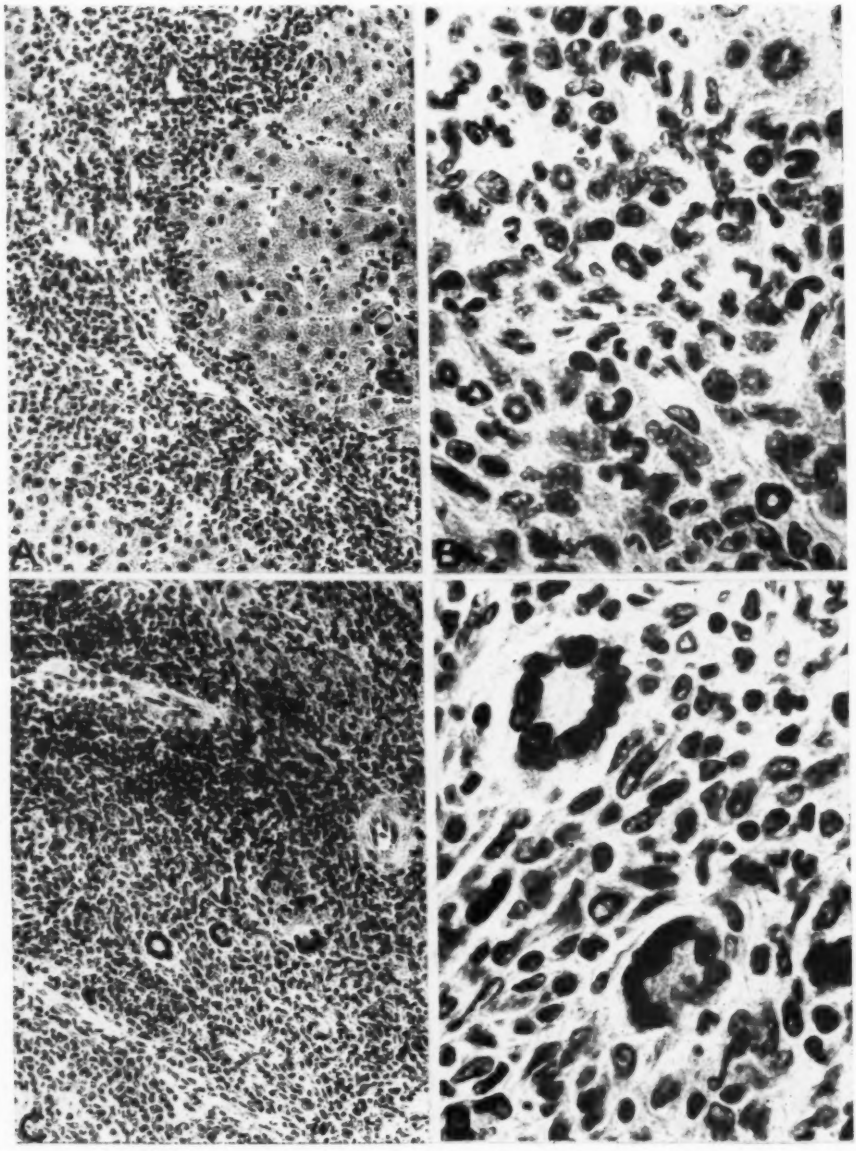

FIGURE 3.—*A* and *B*, Liver from painted mouse Rf 247 showing advanced myeloid infiltration in the portal area of the liver. × 120 and 900, respectively; *C* and *D*, Spleen of mouse 247 showing proliferation of mononuclear cells with multinuclear giant cells in the pulp and myeloid cells. × 150 and 800, respectively. All sections stained with haematoxylin and eosin.

Their nuclei were of the size and shape of monocytes and were fairly uniform. The Rf 247 lesion, we believe, represents a granuloma of unknown character and not a neoplasm. The structure of the lymph node was obscured by masses of cells similar to those seen in the spleen, and granulomalike lesions with the multinuclear giant cells. The marginal sinus was patent. The bone marrow, like that of Rf 245, showed hyperplasia of granulocytes with many eosinophiles. The blood smears from both Rf 245 and 247 taken before death showed a great increase in polymorphonuclear leukocytes, but no leukemia.

Chemical Q_{LA} *Values*

The chemical lactic acid values served not only to establish the absolute Q_{LA} values but also to confirm the manometric Q_A values and possible differences between manometric replicates. Corresponding Q_{LA} and Q_A determinations were always performed on the same sliced material and for an identical period of time. Whereas the chemical Q_{LA} values refer, so far as is known, solely to lactic acid, as indicated by the highly specific method of Barker and Summerson (*19*), the manometric Q_A values refer to net production of any and all acids (including base consumption, if any). It is convenient to consider both the relationship Q_{LA}/Q_A and $(Q_{LA}-Q_A)$.

Table 3 presents all determinations made of Q_A (aerobic, $Q^{O_2}{}_A$; anaerobic, $Q^{N_2}{}_A$) for lymph nodes, spleen, and liver of the mice metabolized (tables 1 and 2) involving three stocks and various leukemic or control groups. On the whole there is the same rough agreement between corresponding paired Q_{LA} and Q_A values as reported in the study of chicken-tumor metabolism (*16*). Without important exception, where replicate determinations are available, the chemical determinations agree qualitatively with the manometric in regard to which replicate value is greatest; quantitatively, however, a comparison of individually paired Q_{LA} and Q_A values may well involve an error of ± 20 per cent, or at times, even more.

For anaerobic conditions the chemical values are equal to, or somewhat greater than, the manometric, i. e., $Q^{N_2}{}_{LA}/Q^{N_2}{}_A$ is equal to, or greater than unity, and $(Q^{N_2}{}_{LA}-Q^{N_2}{}_A)$ is positive. The equality indicates that all the acid produced is lactic acid, and any excess of equality, apart from experimental error, would suggest a production of base as well.

For aerobic conditions $Q^{O_2}{}_{LA}/Q^{O_2}{}_A$ is usually less than unity and $(Q^{O_2}{}_{LA}-Q^{O_2}{}_A)$ is negative. Of especial interest are the aerobic glycolysis values for the livers of the control mice, which are in themselves negative, indicating chemical disappearance of lactic acid at the time at which other acids are being formed, as indicated by the positive manometric $Q^{O_2}{}_A$ values. Nevertheless, in the infiltrated livers (leukemic or leukemoid), the chemical $Q^{O_2}{}_{LA}$ values are no longer negative as in the control livers, because lactic acid is produced by the infiltrated cells more rapidly than the liver can dispose of it. Unfortunately, no chemical data are available for preleukemic mice; hence, no determinations could be made as to whether the chemical $Q^{O_2}{}_{LA}$ values would be negative as in the control group, or positive as in the leukemic or leukemoid groups. In liver, by studying the chemical $Q^{O_2}{}_{LA}$ value, one could have a distinction of sign in characterizing the metabolism of the preleukemic mice as well as the distinction of magnitude indicated in the discussion of the aerobic, manometric glycolysis values earlier. The qualitative experimental question to be answered would be, Do preleukemic livers produce or consume lactic acid (is $Q^{O_2}{}_{LA}+$ or $-$)?

DISCUSSION

In order to assess with proper perspective the experimental results that have been reported in this paper, there is pre-

TABLE 3.—*Comparison of lactic acid production (glycolysis) determined chemically and manometrically in normal, preleukemic, leukemic, and leukemoid mouse tissue (lymph nodes, spleen, liver)*

Nature of tissue	Stock of mice	Mouse No.	Tissue											
			Lymph nodes				Spleen				Liver			
			Glycolysis				Glycolysis				Glycolysis			
			Chemical, Q_{LA}	Manometric, Q_A	$Q_{LA}-Q_A$	Q_{LA}/Q_A	Chemical, Q_{LA}	Manometric, Q_A	$Q_{LA}-Q_A$	Q_{LA}/Q_A	Chemical, Q_{LA}	Manometric, Q_A	$Q_{LA}-Q_A$	Q_{LA}/Q_A
Aerobic:														
Unpainted, controls	Rf	269–274	2.5	2.2	0.3	1.13					0.75	0.51	0.24	
											−.40	.42	−.82	
	Rf	260–264	.9	1.4	−.5	.65	1.6	2.4	−0.8	0.66	−.91	.44	−1.35	
											−.64	.33	−.97	
Painted, leukemic	Rf	246	2.2	2.6	−.4	.85	3.4	4.1	−.7	.83	1.6	2.3	−.7	0.70
Painted, leukemoid (nontransmissible)	Rf	247	4.1	4.5	−.4	.90	3.3	3.9	−.6	.87	.56	1.6	−1.04	.35
											4.0	3.4	.6	1.18
											4.0	3.3	.7	1.22
Spontaneous (unpainted)	C3H/Ak	74	2.9	5.1	−2.2	.57	3.1	4.5	−1.4	.69				
							2.6	4.1	−1.5	.63				
							2.1	4.2	−2.1	.51				
Average			2.5	3.2	−.6	.81	2.7	3.9	−1.2	.70	1.12	1.54	−.42	.86
Anaerobic:														
Unpainted, controls	Rf	269–274	6.7	7.0	−.3	.95	8.6	6.4	2.2	1.35	.26	.25	.01	
			5.0	5.4	−.4	.92								
	Rf/Ak	Mb:89, 91,97,99									.00	.44	−.44	
Painted, negative (preleukemic)	Rf/Ak	Mb: 88, 90, 96									−.05	.37	−.42	
											.31	.62	−.31	
Painted, leukemic	Rf/Ak	Mb 49									.01	.59	−.58	
											3.7	4.3	−.6	.87
	Rf	246	6.6	4.2	2.4	1.58	11.6	6.6	5.0	1.76	3.0	4.1	−1.1	.75
			7.5	4.9	2.6	1.54	10.6	7.6	3.0	1.40	6.4	5.7	.7	1.13
Painted, leukemoid (nontransmissible)	Rf	247	14.3	11.5	2.8	1.25	9.9	8.9	1.0	1.11	5.1	4.4	.7	1.15
			12.6	11.0	1.6	1.15	9.8	8.6	1.2	1.13	14.6	11.0	3.6	1.32
Spontaneous (unpainted)	C3H/Ak	74	10.3	8.0	2.3	1.29	10.0	10.1	−.1	.99	10.8	9.5	1.3	1.14
			9.3	8.5	.8	1.09	7.6	8.5	−.9	.89				
Average			9.0	7.6	1.4	1.22	9.7	8.1	1.6	1.23	4.01	3.75	.26	1.06

sented in table 4 a summary of all available experimental metabolism values on mouse leukemias studied by various investigators to date. These data, collated from the various published papers and from certain unpublished data of Hall[7] have been reduced wherever necessary to standard Q values of comparable experimental significance. In addition, calculations have been made . all these data with respect to the standard quotients customarily employed in analyzing tumor or normal tissue metabolism, viz, absolute and percent Pasteur effects, Meyerhof oxidation quotient, extent quotient, aerobic quotient, and fermentation excess.

Table 4 presents a concise picture of the scope and variety of experimental results available. Thus, in addition to the induced leukemias already described in this paper, there are, as evident in the left-hand column of the table, data available on spontaneous myeloid and monocytic as well as lymphoid leukemias, involving a variety of stocks of mice, and a wide range of spontaneous incidences, and in the case of the myeloid and monocytic leukemias studied by Hall[8] a number of leukemic organs or types of tissue (lymph nodes, liver, spleen, tumor, and cells by estimation). The column headed "Expermental group" shows the many types of experimental problems and factors studied.

Among the metabolic Q values the Q_{O_2} is probably the most constant. The majority of Q_{O_2} values lie between 4 and 7. The Q_{CO_2} values are more variable because of greater difficulty of measurement, and they average 0.75 to 0.85 (=R.Q.) times the Q_{O_2} values. The aerobic fermentation values ($Q^{O_2}{}_A$), although always less in absolute magnitude than the corresponding anaerobic $Q^{N_2}{}_A$, show as a whole the greatest relative variations within groups of comparable interest. The anaerobic $Q^{N_2}{}_A$ values show in general the highest Q values, and they are undoubtedly of greatest interest here from the standpoint of tumor and leukemia metabolism.

In regard to the derived quotients, the almost invariably negative fermentation excess values are of greatest interest. They are negative not because, as in many mouse tumors, the Q_{O_2} values are unusually high, but because the anaerobic glycolysis values, are at best only moderate or low for malignant material. The absolute Pasteur effect is likewise moderate, so that the Meyerhof oxidation quotients are on the whole correspondingly moderate at best, seldom exceeding 5 and ranging largely between 2 and 4.

As regards malignancy of metabolism, the induced leukemias show, on the whole, the least, and the spontaneous leukemias, lines I and M of Victor and Potter, the most. In making any such comparisons, however, it is necessary to keep in mind both the extent of infiltration and the factors affecting it, and also possibly the type of infiltration. It is our impression that extent of infiltration is, generally speaking, a good deal more important than type. It is also important to keep in mind that the Q values, particularly Q_A values, for the various organs reported would often be higher, were it possible to exclude completely the diluting effects of the nonleukemic fractions of the organ.

The Preleukemic State

From the standpoint of studying a possible or definite preleukemic state, the induced leukemias offer great advantages, not only in that the actual appearance of the leukemic state may be more closely governed, but also in that excellent controls of comparable age, with which to judge the definite existence of a preleukemic state, are available in the untreated control animals. A question might

[7] [8] *Hall, V.: See footnote 6.*

TABLE 4.—*Summary of standard metabolism values [1] of mouse leukemias studied by various investigators to date*

Type of leukemia and tissue	Stock of mouse	Eventual spontaneous incidence of leukemia	Experimental group	Investigator	Number of metabolic determinations	Number of animals	Aerobic acid formation (Q^{N}_{2A})	Carbon dioxide production (Q_{CO_2})	Oxygen consumption (Q_{O_2})	Respiratory quotient ($R.Q.=Q_{CO_2}/Q_{O_2}$)	Anaerobic acid formation (Q^{N}_{2A})[1]	Absolute Pasteur effect ($Q^{N}_{2A}-Q^{A}_{2A}$)	Percent Pasteur effect $\left(\frac{100(Q^{N}_{2A}-Q^{A}_{2A})}{Q^{N}_{2A}}\right)$	Meyerhof oxidation quotient $\left(\frac{Q^{N}_{2A}-Q^{A}_{2A}}{Q_{O_2}:3}\right)$	Extent quotient ($Q^{N}_{2A}/Q_{O_2}:3$)	Aerobic quotient ($Q_{O_2}/Q^{N}_{2A}:3$)	Fermentation excess $(Q^{N}_{2A}-2Q_{O_2})$	(1) =
Induced:		*Pct.*																
Liver	Rf/Ak	12	1:	Writers (table 1).														
			Normal		7	11					0.5							
			Preleukemic (painted, negative).		7	13					.6							
			Leukemic		13	8					2.4 (1-4)							
	Rf	3	2:	Writers (table 2).														
			Normal		4	11	0.4	0.8	1.5	0.6	.3			0.4	0.5	0.8	−2.8	
			Preleukemic (painted, negative).		0	0												
			Leukemic		6	3	1.9	3.6	4.5	.78	7.2	5.3	74	3.5	4.8	1.3	−1.8	
			Transmitted		4	3	1.7	3.2	4.2	.76	4.5	2.8	62	2.6	3.2	1.2	−3.9	
			Leukemoid		4	2	3.3	2.8	4.0	.69	9.7	6.4	66	4.9	7.5	2.5	+1.7	
Spleen	Rf/Ak	12	3:	Writers (table 1).														
			Normal		5	11					5.8							
			Preleukemic (painted, negative).		5	13					6.1							
			Leukemic		10	8					6.9							
	Rf	3	4:	Writers (table 2).														
			Normal		8	19	3.2	2.9	4.1	.71	5.7	2.6	44	1.8	4.1	2.3	−2.5	
			Preleukemic (painted, negative).		14	21	4.6	3.1	4.9	.63	5.6	1.2	21	.8	3.6	2.9	−4.0	
			Leukemic		3	2	4.0	4.6	5.5	.84	7.4	3.4	46	1.9	4.1	2.2	−3.6	
			Transmitted		4	3	5.5	4.4	5.7	.77	6.8	1.3	19	.7	3.6	2.9	−4.6	
			Leukemoid		4	2	3.7	4.4	5.8	.77	7.2	3.5	49	1.8	3.8	1.9	−4.4	
Lymph nodes	Rf/Ak	12	5:	Writers (table 1).														
			Normal		5	11					7.5							
			Preleukemic (painted, negative).		6	13					7.4							
			Leukemic		15	8					7.4							
	Rf	3	6:	Writers (table 2).														
			Normal		8	19	2.1	2.9	3.7	.82	5.1	3.0	59	2.5	4.2	1.7	−2.3	
			Preleukemic (painted, negative).		18	27	3.0	2.7	3.4	.80	5.5	2.5	45	2.3	5.0	2.7	−1.3	
			Leukemic		6	3	3.0	3.7	4.8	.79	6.4	3.4	53	2.3	4.3	2.0	−2.8	
			Transmitted		4	3	3.0	4.4	5.0	.91	8.4	5.4	64	3.2	4.9	1.8	−1.6	
			Leukemoid		3	2	4.0	4.8	5.2	.91	8.8	4.8	55	2.8	5.2	2.4	−1.6	

Spontaneous:																	
Lymphatic:																	
Liver	Ak–C3H	40	7: (Spontaneous)	Writers (table 2).	2	2	.9	3.5	5.0	.71	5.6	4.7	84	2.8	3.3	.5	−4.4
					3	2	4.1	5.9	7.9	.75	12.0	7.9	66	3.0	4.6	1.6	−3.6
Spleen					3	2	4.1	4.7	6.4	.73	10.3	6.2	60	2.9	4.9	2.0	−2.5
Lymph nodes																	
Lymph nodes	Ak	70	8:	Hall and Furth (*12*).													
			Normal		[2] 10	2.0		5.5	6.3	.87	4.7	2.7	57	1.3	2.3	1.0	−7.9
			Leukemic		[2] 39	3.6		5.2	6.0	.86	10.5	7.0	66	3.5	5.3	1.8	−1.5
			Transmitted		[2] 13	3.2		4.7	5.7	.82	7.6	4.4	58	2.3	4.0	1.7	−3.8
			Incidental factors:														
			9. Sex:														
			Male			2.6			6.6		11.4	8.8	77	4.0	5.2	1.2	−1.8
			Female			2.5			6.1		11.0	8.5	77	4.2	5.4	1.2	−1.2
			10. Age (days):														
			125–224		5	2.6			5.7		11.7	9.1	78	4.8	6.2	1.4	+.4
			225–299		12	2.9			5.9		11.5	8.6	74	4.3	5.9	1.5	−.3
			300–399		13	3.9			5.5		8.4	4.6	54	2.5	4.5	2.1	−2.7
			400–550		6	3.0			6.0		9.5	6.5	69	3.4	5.0	1.5	−2.4
			11. Percentage of malignant cells:														
			A (95–100)		3	2.8			6.7		13.1	10.3	78	4.4	5.9	1.3	−.3
			B (90–95)		15	3.1			6.8		12.3	9.3	75	4.1	5.4	1.4	−1.3
			C (70–90)		10	4.0			6.1		9.0	5.0	55	2.5	4.5	2.0	−3.0
	RAk	12	12:														
			Leukemic		[3] 51	1.3		6.2	6.4	.97	8.5	7.2	85	3.4	4.0	.6	−4.3
			Transmitted		[3] 15	2.5			6.3		10.4	7.9	76	3.8	5.0	1.2	−2.2
			Incidental factors:														
			13. Sex:														
			Male			1.3			6.5		7.9	6.5	83	3.0	3.6	.6	−5.2
			Female			1.3			6.5		8.6	7.3	85	3.4	4.0	.6	−4.4
			14. Age (days):														
			125–224		6	1.1			6.4		9.8	8.8	89	4.1	4.6	.5	−2.9
			225–299		19	1.2			6.4		8.4	7.3	86	3.4	4.0	.5	−4.3
			300–399		16	1.9			6.3		7.9	6.0	76	2.8	3.7	.9	−4.8
			400–550		6	2.6			6.5		7.6	5.0	66	2.3	3.5	1.2	−5.4
			15. "Percentage of heredity":														
			100 Ak (leukemia-susceptible)		39	3.6			6.0		10.5	7.0	66	3.5	5.3	1.8	−1.5

[1] Values (including $Q_{N_{2A}}$) of the writers are for first 2 hours after preliminary equilibration in thermostat; values of other investigators are largely or entirely for about first hour. Q_{O_2} values of other investigators were, unlike those of the writers, not obtained in the same CO_2–bicarbonate medium employed to measure the glycolyses, but in a CO_2–free, phosphate-buffered medium. The observations of Victor et al. were carried out with the Fenn respirometer technique, whereas all other investigators employed Warburg or Summerson manometer techniques. As discussed in the section on Methods, in the case of lymph nodes, the percent initial dry weight has been arbitrarily taken to be 18 percent in our investigations, 19.1 percent in the work of Hall and Furth (*12*), and 20 percent in the work of Victor et al. Q values based on 20-percent dry weight would thus be 10 percent (2 parts in 20) lower than those based upon 18 percent, if 18 percent were the correct figure. Apart from these various differences the general procedures employed by all investigators were very much the same; thus, 0.2 percent glucose was added to all liquid media, unless otherwise specified; 5 percent CO_2 in all gas phases was used in measuring glycolysis; initial and not final dry weight percentages were employed (for discussion of desirability of using dry weight taken before metabolic measurement see (*16, p. 227*) and others referred to therein, and Victor and Wintersteiner (*10, p. 263*), and footnote 4, table 2); and temperatures of 37.5° to 38° C. were employed. All the animals employed by the present writers were 3 to 6 months old at most, whereas in the case of other investigators (*12, 8, 4*) leukemic animals were middle-aged or old. In the A, B, C classification used by Hall and Furth (*12*), none of the lymph nodes employed by the present writers attained more than class B or C status.

[2] In general, determinations for each mouse were made separately, but seldom for all metabolic values simultaneously, owing to lack of material; thus, the number of determinations for any one Q value is a variable fraction (⅓ to ½) of the total number of mice.

[3] RAk mice include all hybrids from any type of cross between Rf and Ak mice, whether the F_1 hybrid or otherwise. The Rf/Ak mice are the F_1 hybrids from the Rf female (including any subline, as Rfk or Rfl, etc.) and Ak male (including any subline, as Akl, Akm, etc.).

TABLE 4.—*Summary of standard metabolism values of mouse leukemias studied by various investigators to date*—Continued

Type of leukemia and tissue	Stock of mouse	Eventual spontaneous incidence of leukemia	Experimental group	Investigator	Number of metabolic determinations	Number of animals	Aerobic acid formation (Q^{N}_{2A})	Carbon dioxide production (Q_{CO_2})	Oxygen consumption (Q_{O_2})	Respiratory quotient ($R.Q.=Q_{CO_2}/Q_{O_2}$)	Anaerobic acid formation (QN_{2A})[1]	Absolute Pasteur effect ($QN^{2}_{A}-Q^{O_2}_{A}$)	Percent Pasteur effect $\left(\frac{100(QN^{2}_{A}-Q^{O_2}_{A})}{Q^{O_2}_{A}}\right)$	Meyerhof oxidation quotient $\left(\frac{QN^{2}_{A}-Q^{O_2}_{A}}{Q_{CO_2}/3}\right)$	Extent quotient $(QN^{2}_{A}/Q_{O_2}/3)$	Aerobic quotient $(Q^{O_2}_{A}/Q_{O_2}/3)$	Fermentation excess ($F=Q^{O_2}_{A}-2Q_{O_2}$)
		Pct.	88 Ak (12 R, nonsusceptible)			13	0.5		6.1		7.6	7.1	94	3.5	3.7	.2	−4.7
			75 Ak (25 R)			22	1.3		6.4		7.9	6.6	84	3.1	3.7	.6	−4.8
			50 Ak (50 R)			12	2.0		6.8		9.0	7.0	78	3.1	4.0	.9	−4.6
Spontaneous: Lymphatic: Lymph nodes	89		16:	Victor et al. (4).													
			Normal (animals 6–8 weeks old).		12	48	[2]2.1		4.8		[2]6.4 (− .3)	4.3	67	2.7	4.0	1.3	−3.2
		Rare	Preleukemic (animals 6–10 months old).		13	26	1.8	4.1			6.6 (+ .5)	4.8	73	3.4	4.7	1.3	−1.6
	Dbr		17:														
		Rare	Normal (animals 6–8 weeks old).		10	30	2.5		6.0		6.3 (− .9)	3.8	60	1.9	3.1	1.3	−5.7
			Preleukemic (animals 6–10 months old).		12	24	1.4		4.3		6.0 (+ .3)	4.6	77	3.3	4.3	1.0	−2.6
	Bagg-albino		18:														
		Rare	Normal (animals 6–8 weeks old).		12	48	2.0		5.0		5.9 (−1.0)	3.9	66	2.3	3.5	1.2	−4.1
			Preleukemic (animals 6–10 months old).		13	30	1.7		3.9		5.4 (− .5)	3.7	69	2.8	4.1	1.3	−2.4
	Storrs-Little.		19:														
		1	Normal (animals 6–8 weeks old).		17	68	2.4		5.6		5.9 (− .9)	3.5	59	1.8	3.1	1.3	−5.3
			Preleukemic (animals 6–10 months old).		14	42	1.7		4.5		5.3 (− .3)	3.6	68	2.4	3.5	1.1	−3.7
			20. Transmitted:	Victor et al. (3).													
			Line M-liver on stock C58		12	24	8.4		5.8		18.2 (−1.6)	9.8	54	5.2	9.6	4.4	+6.6
			Line M-liver on stock Storrs-Little from C58.		10	20	4.7		6.0		11.4 (−1.8)	6.7	59	3.3	5.7	2.3	−.6
			Line M-liver on stock C58 from Storrs-Little.		5	10	8.8		5.4		18.9 (−1.4)	10.1	53	5.6	11.0	4.9	+8.1
			Line I on stock C58 (264–275th transfer [6]).		10	20	7.8		5.5		16.6 (−1.4)	8.8	53	4.9	9.2	4.3	+5.6
			Line I on stock Storrs-Little from C58.		10	20	4.4		6.5		10.3 (− .9)	5.9	57	2.7	4.7	2.0	−2.7
			Line I on stock C58 from Storrs-Little.		6	12	7.0		5.6		18.5 (−1.6)	11.5	62	6.0	9.7	3.8	+7.3

[4] Essentially all the $Q^{O_2}{}_A$ values of Victor et al. must be subjected to 2 small usually compensating corrections, if they are to be consistent in experimental significance with $Q^{O_2}{}_A$ values of other investigators reported in this table. It would be worth while to consider these two corrections in adequate detail. Both corrections come about from the fact that in the respirometer technique employed by Victor et al. certain corrections were based upon measurements in aerobic control vessels containing no glucose in the medium bathing the tissues. These control vessels measured the difference between the endogenous oxygen consumption on the one hand and CO_2 production in both respiration and glycolysis on the other. In the earlier papers of Victor et al. the basic assumption was implicit that these endogenous metabolic processes would proceed at the same rate in the absence of glucose as in its presence. According to modern views, however, for which a great deal of evidence has accumulated, the small endogenous no-glucose glycolysis is very probably largely suppressed in the presence of glucose, so that glycolysis occurring in the presence of glucose probably represents essentially the true glycolysis of added glucose per se, and it is unnecessary or undesirable to subtract the glycolytic acid production in the absence of glucose from that in its presence. The use of excess glycolysis values was based in general on an early concept of Meyerhof (*13, pp. 152–155*), and in particular on the original ("unimproved") method developed, but immediately discarded by Warburg (*23*) in favor of his "improved", two-vessel method (*24*). The almost invariable general practice in metabolic studies for well over a decade has been to omit from consideration the very doubtfully significant endogenous values. The aerobic glycolysis values of Victor et al. should, to be consistent with values in the literature, be increased a little (possibly 0.3 to 1.0) on the basis of the foregoing considerations. On the other hand, as indicated by Victor and Potter (*8, pp. 556–558; 9, pp. 573–574*) and see footnote 5, this table, the aerobic no-glucose control they employed had the effect of underestimating the R. Q. value to which (as they indicated (*8*), and also in the experience of many investigators) the R. Q. is ordinarily raised by perhaps 0. 1 (e. g., from 0. 75 to 0. 85) when glucose is added to the medium. Therefore, the difference between oxygen consumed and carbon dioxide produced in respiration was almost certainly less in the experimental vessel with glucose than in the endogenous control vessel, and as Victor and Potter indicate for particular cases (*8, p. 558*), the original, aerobic, no-glucose control corrections applied by them in essentially all their reported $Q^{O_2}{}_A$ values were overcorrected by amounts of 0.3, 0.8, and 1.1 for line M-liver, line I, and normal nodes, respectively, amounting to about 3, 10, and 50 percent increases in the reported $Q^{O_2}{}_A$ values of 8.4, 7.8, and 2.1.

In a much earlier publication, Victor and Wintersteiner (*10, p. 586*) concluded that a wide difference in R. Q. (even 1.00 as compared with 0.70) between vessels with and without glucose would make a negligible difference in $Q^{O_2}{}_A$. This deduction was implicitly corrected several papers later (*8, p. 558*).

It will be seen from the foregoing that both the "excess glycolysis" correction and the overcorrected "R. Q." correction, as they may be called, were not experimentally separated, since they were both in a sense lumped together in the same experimental aerobic, no-glucose control vessel. Fortunately, each correction appears to be small and, in fact, compensating in both sign and magnitude, on the average at least, even if from tissue type to tissue type this may not be so true. It would appear that the quantitative effects of glucose in suppressing endogenous glycolysis on the one hand and raising the R. Q. on the other (with increased CO_2 production in relation to O_2 consumption) just about counterbalance, so that the aerobic, no-glucose control may with few exceptions be applied. It is probable that the numerical corrections, examples of which are given in the parenthesized values reported in the $Q^{N_2}{}_A$ column (see footnote 5, this table) are, if anything, a little large, that is, the R. Q. overcorrection is probably a little larger than the implicated correction for the endogenous glycolysis; the $Q^{O_2}{}_A$ values of Victor et al., as reported by them and reproduced in this table, are thus possibly too large by a small fraction of Q unit, on the average. Victor and Potter (*8, p. 558*) came to the same qualitative conclusion. We believe the average error is less than they presumed, owing to the compensating corrections for endogenous glycolysis that have been introduced into the considerations. In any event, the larger the $Q^{O_2}{}_A$ value, the smaller is the percentage uncertainty, and it is only for $Q^{O_2}{}_A$ values of about 3 to 2 or less that any really important relative or absolute uncertainty could be concerned.

The aerobic, no-glucose control employed by Victor et al. has the merit of taking some experimental account (subject to the foregoing uncertainties) of the fact that the R. Q. of most tissues, and in particular those of interest in leukemic studies (with the known single exception of line A (*8, pp. 557–558*)) are definitely less than unity. In many important instances in the early tissue metabolism literature (e. g., Warburg (*24*); Murphy and Hawkins (*25*); and cf. Crabtree (*26, pp. 537–540*) and Burk (*15, p. 242*)), the assumption was made in determining aerobic glycolysis that the R. Q. was unity, whereas, since the R. Q. was in fact less than unity, $Q^{O_2}{}_A$ values as reported were smaller than they actually were, by an amount equal to Q_{O_2} (1—R. Q.), and the respective Q_{CO_2} values were correspondingly larger; for any rare cases where the R. Q. was greater than unity, the $Q^{N_2}{}_A$ values would then be less than reported and the Q_{CO_2} values greater, by this algebraic quantity. The most interesting case involving such needed corrections for aerobic glycolysis calculations were probably those where the $Q^{O_2}{}_A$ values were reported as zero, whereas for R. Q. values of 0.8 to 0.9 almost certainly involved, the $Q^{O_2}{}_A$ values would really have been not zero but positive by 0.5 to 2 units. R. Q. corrections for aerobic glycolysis are entirely unnecessary, and taken care of experimentally, when the well-known Summerson, Dixon-Keilin, or Dickens-Greville differential manometers are employed. The volumetric technique employed by Victor et al. arrived at approximately the same end, subject, however, to the two uncertainties described.

[5] Parenthetical values here and below in this column are corrections applied to the $Q^{N_2}{}_A$ (and $Q^{O_2}{}_A$) values originally reported by Victor et al. (*3–6, 9–10*). As indicated by Victor and Potter ((*9, p. 576*; *7, p. 228*)), these earlier reported $Q^{N_2}{}_A$ values had been calculated to include an unnecessary addition of the aerobic, no-glucose control (see footnote 4 of this table), that happened to express the amount by which the aerobic carbon dioxide production was less than the oxygen consumption; such a control involving aerobic conditions and the absence of glucose obviously has no bearing on the experimental determination of anaerobic glycolysis in the presence of glucose. This aerobic no-glucose control was used in place of a control first recommended by Warburg (*24*), but shortly afterward discarded by him, namely, an anaerobic, no-glucose control which would measure any endogenous anaerobic glycolysis. The subtraction of this anaerobic, no-glucose control from the anaerobic, glucose, experimental vessel value would give a so-called excess anaerobic glycolysis value, but Victor[11] did not make these measurements, and, as may be seen in footnote 4, this table, we are not interested here in such values, since they involve the assumption that glucose does not largely inhibit endogenous glycolysis. Victor very kindly supplied us with the hitherto unpublished numerical values he had observed in each individual volumetric determination; the values given in parentheses in the $Q^{N_2}{}_A$ column of this table are the averaged corrections which apply to each respective, average $Q^{N_2}{}_A$ determination. The $Q^{N_2}{}_A$ values given here, adjacent to the parenthesized corrections, have been subjected to these corrections. Correction values in double parentheses have, in the absence of available original data with respect to them, been assigned in accordance with expectation based on values in single parentheses, which are of the order of 1 for $Q^{N_2}{}_A$ values up to 10, and 1.5 for $Q^{N_2}{}_A$ values somewhat greater than 10. With these corrections of certain of the reported $Q^{N_2}{}_A$ data of Victor et al., all the $Q^{N_2}{}_A$ values reported in this table now refer consistently to anaerobic glycolysis (acid production) obtained in the presence of 0.2 percent glucose, and uncorrected for observations taken either aerobically or in the absence of glucose, or both, as discussed in footnote 4.

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TABLE 4.—*Summary of standard metabolism values of mouse leukemias studied by various investigators to date*—Continued

Type of leukemia and tissue	Stock of mouse	Eventual spontaneous incidence of leukemia	Experimental group	Investigator	Number of metabolic determinations	Number of animals	Aerobic acid formation ($Q^{N_2}_{A}$)	Carbon dioxide production (Q_{CO_2})	Oxygen consumption (Q_{O_2})	Respiratory quotient ($R.Q.=Q_{CO_2}/Q_{O_2}$)	Anaerobic acid formation (QN_{2A})[1]	Absolute Pasteur effect ($Q^{N_2}_{A}-Q^{O_2}_{A}$)	Percent Pasteur effect $\left(\frac{100(Q^{N_2}_{A}-Q^{O_2}_{A})}{Q^{N_2}_{A}}\right)$	Meyerhof oxidation quotient $\left(\frac{Q^{N_2}_{A}-Q^{O_2}_{A}}{Q_{O_2}/3}\right)$	Extent quotient ($Q^{N_2}_{A}/Q_{O_2}/3$)	Aerobic quotient ($Q^{O_2}_{A}/Q_{O_2}/3$)	Fermentation excess ($\Gamma=Q^{N_2}_{A}-2Q_{O_2}$)
	C58	*Pet.* 90	21:	Victor et al. (*4, 5,8*)													
			Normal (animals 6–8 weeks old).		12	48	2.1	4.8	5.5	.87	5.0 (− .8)	2.9	58	1.6	2.8	1.2	−6.0
			Preleukemic (animals 6–9 months old).			28	3.1		4.8		6.6 (−1.2)	3.5	53	2.2	4.1	4.9	−3.0
			Spontaneous (animals 8–15 months old).	Victor et al. (*5*)	23	23	4.3	5.2	5.6	.92	8.8 (−1.5)	4.5	51	2.4	4.6	2.9	−2.4
			Transmitted (animals 6–8 weeks old):	Victor et al. (*8,10*).													
			Line A		11	33	1.9	6.3	6.4	.98	7.7 (− .6)	5.8	75	2.8	3.7	8.8	−5.1
			Line I (243–247th transfer [6])		9	18	5.6	4.6	5.5	.84	11.8 (−1.0)	6.2	52	3.4	6.6	3.1	+.8
			Line M-spleen D		12	24	6.2		6.7		13.3 (−2.0)	7.1	53	3.2	6.0	2.8	−.1
			Line M-liver		12	24	8.4	4.7	5.8	.81	18.2 (−1.6)	9.8	54	5.2	9.6	4.4	+6.6
			22:	Victor et al. (*6*).													
			Transmitted (animals 6–8 weeks old) line M-liver.		9		8.4		5.3		16.9((−1.5))	8.5	50	4.7	9.4	5.0	+6.3
			Transmitted (animals 6–8 months old) line M-liver.		11		6.9		4.6		14.9((−1.5))	8.0	54	5.3	9.9	4.5	+5.7
			Incidental factors:														
			23. Age of host:[7]														
			Transmitted (young-young) Line O.				2.6	4.6	5.3	.89	8.7((−1.0))	6.1	70	3.4	4.8	1.6	−1.9
			Transmitted (old-young) line O.				2.7	4.2	5.0	.84	8.3((−1.0))	5.6	68	3.3	4.9	1.6	−1.7
			Transmitted (young-old) line O.				2.4	3.9	4.8	.81	7.5((−1.0))	5.1	68	3.2	4.7	1.5	−2.1
			Transmitted (old-old) line O				2.2	3.6	4.7	.77	7.1((−1.0))	4.9	69	3.1	4.4	1.4	−2.3
			24. Time after inoculation:	Victor et al. (*7*).													
			Line I (520–532nd transfer):														
			Peripheral nodes:														
			Before inoculation		14		2.3		4.3		5.2	2.9	55	2.0	3.6	1.6	−3.5
			1 day after		16		2.0		4.5		4.0	2.0	49	1.3	2.6	1.4	−5.1
			2 days after		10		2.4		3.8		2.9	.6	19	.4	2.3	1.9	−4.7
			2 days after (infiltrated +).		11		2.3		4.8		4.0	1.7	42	1.1	2.5	1.5	−5.6
			3 days after (infiltrated + to ++).		18		5.5		4.6		7.6	2.2	28	1.4	5.0	2.7	−1.6
			4 days after (infiltrated ++ to ++++).		9		4.5		4.7		9.2	4.7	51	3.0	5.8	2.9	−.7
			Mesenteric nodes:														
			4 days after (infiltrated		9		4.5		4.9		10.4	6.0	57	3.6	6.4	2.7	+.6

+++ to ++++).													
Line A:													
Peripheral nodes:													
Before inoculation		14	2.2	3.8	4.5	2.3	51	1.8	3.5	1.7	−3.1		
1 day after		16	1.7	4.2	4.6	2.9	63	2.1	3.3	1.3	−3.7		
2 days after		16	1.8	4.1	3.6	1.8	49	1.3	2.7	1.3	−4.6		
3 days after		11	1.9	4.2	3.2	1.3	41	1.0	2.3	1.3	−5.2		
4 days after		4	2.3	4.2	4.3	2.0	47	1.5	3.1	1.6	−4.0		
5 days after		2	2.1	3.0	3.2	1.2	36	1.2	3.2	2.1	−2.8		
6 days after		2	2.2	4.2	4.1	1.9	47	1.4	2.9	1.5	−4.4		
4 days after (infiltrated +).		2	2.7	4.1	6.7	4.0	59	2.9	4.8	2.0	−1.6		
5 days after (infiltrated + to ++).		4	4.0	4.3	10.7	6.7	63	4.7	7.4	2.8	+2.1		
6 days after (infiltrated +).		4	3.5	3.9	6.6	3.1	47	2.4	5.1	2.7	−1.2		
Mesenteric nodes:													
2 days after		5	1.7	4.3	3.5	1.8	52	1.3	2.5	1.2	−5.2		
3 days after		3	2.5	4.2	3.4	.9	27	.6	2.4	1.8	−5.1		
3 days after (infiltraten+).		2	2.6	3.4	6.6	4.1	61	3.6	5.9	2.3	−.1		
6 days after (infiltrated +++to++++).		6	3.4	4.1	11.4	8.0	77	5.9	8.4	2.5	+3.3		
25: Influence of medium:	Victor et al (9, 11).									3.2	−4.3		
Normal cells on Ringer's solution.				4.6	4.9					2.5	−6.5		
Normal cells in normal serum.				5.5	4.5					6.4	+.6		
Line I cells in Ringer's solution				4.9	10.5					5.6	−.7		
Lines I cells in normal serum				6.1	11.6					5.8	−.3		
Line I cells in immune serum				5.6	10.9					4.3	−3.6		
Line cells in line I serum				6.2	8.8								

[1] It is by no means clear why the glycolysis values of line I on C58 mice were markedly lower for the 243d–247th transfers (metabolized December 1932) than for the 264th–275th transfers (metabolized February–April 1933). No explanation for this discrepancy appears in the two papers concerned (*10, 3*) although in later papers (*8, 7*) the latter, higher values are cited as though apparently then preferred. Nevertheless, in the latest set of data taken (*7*) the former lower values were again obtained for line I on C58 mice. Were the lower values really correct and the higher ones not, the conclusions (*3*) would be markedly affected, and, contrariwise regarding interpretations (*10, 7*), if the higher glycolysis values are correct; that both are correct may be possible, but certainly not readily understandable or explained as yet. Victor has commented recently:[a] "We are as certain as to the values recorded in the loci cited as we are confused as to their cause. Later unpublished experiments were undertaken to discover the cause of metabolic changes that may occur in the history of individual transmission lines. By starting with spontaneous cases and transmitting through fifteen transfers marked changes occur in virulence without corresponding changes in metabolism. Occasional changes in cell type occur in transmission lines, and this may be more closely related to metabolism. We have not been fortunate enough to have observed the metabolism of line I or line A through a period in which the cell type changed." To the foregoing may be added the observation that the Qo_2 values reported (*8*) for normal nodes of young mice of stock C58, and also for line I (February and December 1935) and line M-liver (October 1933) were 18 to 33 percent lower than reported (*3, 10*) (Qo_2 values of 4.5, 4.3, and 3.9, or 1.0, 1.2, and 1.9 less than 5.5, 5.5, and 5.8, respectively). These changes in Qo_2 are similar to the changes in glycolysis just referred to, except that they refer to two sets of transmission lines and normal nodes as well. This suggests that something unrecognized in technical procedure, such as dry-weight determination, mode of slicing, etc., may be involved, rather than an actual change in metabolism of the respective transfers. Data are given (*8*) showing that omission of glucose from the medium increases the Qo_2 for normal nodes and nodes of animals bearing line I, line M-liver, line M-spleen, and line A by 8 to 25 percent, the R. Q. values of normal nodes and line I by about 20 percent (line M-liver nodes not at all). These changes of Qo_2 induced by omission of glucose presumably have no connection with the changes in Qo_2 and Q_A reported for different transfer series of transmissions or normal nodes, since in these latter instances 0.2-percent glucose was invariably present, according to the description of the technique given (*3, 10, 8*). Victor and Potter (*9, p. 576*) have shown that serum, as compared with Ringer's solution, raises the Qo_2 by approximately 1 unit in 4 to 5, in the cases of normal and line I lymphoid cells.

[a] This experiment was devised to test the reversibility of the effect of host age. The cells of the spleen of a C58 mouse with spontaneous lymphatic leukemia (line O) were inoculated into young (6–8 weeks old) and old (6–9 months old) mice, and further transmissions from both these groups were made into young and old mice; hence young-old indicates a passage into young animals with a concluding passage into old mice, etc.

TABLE 4.—*Summary of standard metabolism values of mouse leukemias studied by various investigators to date*—Continued

Type of leukemia and tissue	Stock of mouse	Eventual spontaneous incidence of leukemia	Experimental group	Investigator	Number of metabolic determinations	Number of animals	Aerobic acid formation ($Q^{N_2}_A$)	Carbon dioxide production (Q_{CO_2})	Oxygen consumption (Q_{O_2})	Respiratory quotient (R.Q.=Q_{CO_2}/Q_{O_2})	Anaerobic acid formation ($Q^{N_2}_A$)[1]	Absolute Pasteur effect ($Q^{N_2}_A - Q^{O_2}_A$)	Percent Pasteur effect $\left(\frac{100(Q^{N_2}_A - Q^{O_2}_A)}{Q^{N_2}_A}\right)$	Meyerhof oxidation quotient $\left(\frac{Q^{N_2}_A - Q^{O_2}_A}{Q_{O_2}/3}\right)$	Extent quotient ($Q^{N_2}_A/Q^{O_2}/3$)	Aerobic quotient ($Q^{O_2}_A/Q^{O_2}/3$)	Fermentation excess ($\Gamma = Q^{N_2}_A - 2Q_{O_2}$)
Myeloid:[2]		*Pct.*	26:	Hall[3]													
Lymph nodes	Ak	70	Normal		10		2.0	5.2	6.0	.87	4.5	2.6	57	1.3	2.2	1.0	−7.5
	Rf	3	Nonmalignant myeloid infiltration									3.3	68	2.0	3.0	.9	−4.9
			Chloroleukemia, strain Slb 351		6		1.5	5.1	4.8	.86	4.8	5.2	59	2.3	4.5	2.2	−3.3
	Sl	20	Myeloid, strain Akh 106		23		2.8	5.6	6.8	.86	10.3	4.9	64	2.6	4.1	1.5	−3.9
	Ak	70			4	4		5.8	5.8		7.7						
Spleen	Ak		27:														
	Rf	70	Normal		10		3.1	5.7	6.6	.87	6.8	3.7	55	1.7	3.1	1.4	−6.5
		3	Nonmalignant myeloid infiltration		7		2.3	3.7	4.6	.81	4.9	2.6	53	1.7	3.2	1.5	−4.2
			Chloroleukemia, strain Slb 351		25		6.3	5.9	6.9	.86	11.8	5.5	47	2.4	5.1	2.1	−2.1
	Sl	20	Myeloid, strain Akh 106		4	4	4.8		7.0		9.5	4.7	50	2.0	4.1	2.1	−4.5
	Ak	70															
Liver	Ak		28:														
		70	Myeloid, strain Akh 106		1		6.5		6.0		7.0	1.7	21	.8	3.5	3.3	−5.0
Tumor (subcutaneous)			29:														
	Ar	15	Malignant myelocytic, strain Ar 117		1		2.8	3.1	3.4	(.9)	6.9	4.1	59	3.7	6.3	2.5	+.1
Myeloid cells, estimated:[3]			30:														
Spleen	Rf	3	Nonmalignant myeloid infiltration				3.1		6.6		4.8	1.7	38	.8	2.2	1.4	−8.5
			Chloroleukemia, strain Slb 351				6.8		7.0		12.7	5.9	46	2.5	3.7	2.9	−1.3
Tumor	Sl	20	Malignant myelocytes, strain Ar 117				5.8		7.3		14.5	8.7	60	3.6	6.0	2.4	−.1
	Ar	15															
Monocytic:	Rf	3	31: Strain Rfb 385.														
Spleen[10]					6		3.2		5.1		7.8	4.6	59	2.7	4.7	1.9	−2.3
Liver[10]					9		2.2		3.9		4.6	2.4	52	1.8	3.5	1.7	−3.3
Tumor					4		3.6		3.4		5.2	1.6	31	1.4	4.6	3.2	−1.5
Monocytic cells, estimated:[3]																	
Spleen					4		3.9		8.0		11.2	7.4	66	2.8	4.2	1.5	−4.7
Liver					4		7.2		9.8		17.0	9.8	58	3.0	5.2	2.2	−2.6
Tumor					4		5.7		5.1		8.2	2.5	31	1.5	4.8	3.4	−2.0

[1] Data supplied by Dr. Victor Hall from papers in preparation or in press, based on data taken by him several years ago in the Department of Pathology, Cornell University Medical College. Lines Slb 351, Akh 106, Ar 117 (now extinct), and Rfb 385 have already been described (*27, 71, 28, 29*). Stocks Ar and Sl are now extinct.

[2] Estimated values for infiltrated cells calculated by Dr. Hall on the basis of histologic observations. As the basis of calculation, the increase in metabolism of an organ containing the abnormal cells has been related to the fraction of such abnormal cells in the organ containing them. Such calculations are admittedly empirical, and of approximating significance only. When the proportion of malignant cells was less than 30 percent the data were excluded by Dr. Hall, it being felt that the estimates were then too unreliable.

[3] Values of liver and spleen included for cases of monocytic leukemia only when liver and spleen were definitely infiltrated.

[4] Victor, J.: Private communication (1942).

remain whether the leukemogenic agents might produce changes prior to the onset of leukemia that might not, or could not, be definitely connected causally with the leukemia. An excellent example of this sort of possible situation would be the marked cirrhosis and fatty degeneration of liver that commonly precedes hepatoma formation in rats fed *p*-dimethylaminoazobenzene in diets that do not protect against tumor formation; by suitable dietary regime (involving, e. g., cystine, choline, riboflavin), it is possible to obtain hepatoma formation without such accompanying liver damages. Also, preleukemic metabolic changes might be difficult to detect, if only a relatively small number of preleukemic or malignant cells were involved, and measurement of their activity were swamped by other cells. On the other hand, it is possible that a large number of cells are altered metabolically even though appearing to remain normal histologically. A major purpose of the present study has been to obtain information on this point.

Heretofore, Victor and Potter (*3–8*) have made two different attempts to study the metabolism during the preleukemic period, one involving the period before the development of spontaneous leukemia, the other before successful transmission after inoculation. In the first instance Victor and Potter (*3, 4, 5, 8*) made a metabolic comparison between nodes taken from young mice (6–8 weeks old) with nodes taken from mice 6 to 10 months old at a period when spontaneous leukemias were beginning to develop in the strain employed. According to their published values (table 4, $Q^{N_2}{}_A$, including parenthetical additions) there were four strains (89, Dbr, Bagg-albino, Storrs-Little) in which the spontaneous incidence was very low and in which the aerobic and anaerobic glycolyses were found to decrease in the older nodes; whereas, in the strain tested with the high incidence (C58) the glycolyses were found to increase in the older nodes. It is perhaps questionable whether such older nonmalignant nodes are better referred to as preleukemic or as simply older nodes, especially in strains where the eventual incidence of spontaneous leukemia is by no means 100 percent. The difficulty here is that there is no suitable (untreated) control with which to compare the possible preleukemic state of the nodes before onset of leukemia, even though comparison can be made after onset of leukemia. Finally, the $Q^{N_2}{}_A$ values of Victor and Potter as revised in table 4 now indicate no significant glycolytic change between young mice (6–8 weeks) and older, possibly preleukemic mice (6–10 months) for the four low-incidence strains, and in the case of the high-incidence C58 strain the increase in the older mice is reduced also, but not quite to within the limits of statistically significant difference. Later transmission experiments (*7*) indicated that some of the older mice were probably leukemic. The Q_{O_2} of the older mice in the four low-incidence strains was lower than in the young mice, but this was also true in the high-incidence C58 strain. Victor and Potter (*7*) also made a study of lymph-node metabolism just before and immediately after intraperitoneal inoculation of transmission lines, and made numerous determinations on material taken at biopsy and at death, which occurred only some 4 to 10 days after inoculation. In the second case the time period involved was so short, rather than so prolonged as in the first case, as again to make designation as a preleukemic state seem questionable. Since multiplication of leukemic cells begins with the time of inoculation, these mice should be regarded as leukemic in a latent period.

The data of Victor and Potter on spon-

[11] Victor, J.: Private communication (1942).

taneous leukemias may be interpreted as indicating (as did our data on induced leukemias) that there is no definite indication, prior to the sudden onset of frank leukemia, of metabolic changes in the direction of malignancy. There is, to be sure, an apparent increase in the aerobic glycolysis in the C58 strain, as in our data in table 2, but the increases are just definitely significant, and they may bear no relationship to a definite preleukemic state.

Future Investigations

The following particular experimental data on aspects uncertain, incompleted, or not studied in this paper, would be desirable in any further extension of this investigation: (1), Preleukemic livers, Q_{O_2} and Q_{CO_2} values, and $Q^{O_2}{}_A$ values, and particularly the sign of $Q^{O_2}{}_{LA}$; (2), transmitted leukemias, the various Q values, leukemoid (nonmalignant extramedullary myelopoiesis) infiltrations, especially in liver, preleukemic and normal; (3), confirmation of $Q^{O_2}{}_A$ increases in leukemic and leukemoid lymph nodes and spleens as contrasted with normal and preleukemic lymph nodes and spleens; (4), further confirmation of absence of significant $Q^{N_2}{}_A$ increases in painted preleukemic or leukemic mice as compared with unpainted mice; (5), comparison of $Q^{N_2}{}_A$ values of livers of Rf and Rf/Ak leukemias transmitted in Rf/Ak stock to determine whether the greater values for Rf as compared with Rf/Ak found in the present experiments represent a genetic difference or some one of the incidental factors already discussed (studies to involve the several serial transfers through primary, chronic, and acute phases); (6), a study of spontaneous leukemias as a function of time of appearance with metabolic analyses on biopsy material before and after onset (including normal nodes of old mice), to see in particular whether higher $Q^{N_2}{}_A$ and other metabolic values, increased $Q^{N_2}{}_A$ and other Q values as compared with the induced leukemias may be the result of the slow, and much more extended period of development; (7), the introduction of the succinate and paraphenylenediamine Q_{O_2} substrates as developed by Craig, Bassett, and Salter (*30*); (8), further work on the percent initial dry weight of lymph nodes, normal and diseased; (9), and, in general, further attempts to distinguish metabolically between different types of leukemic cells, especially in relation to possible comparison with human leukemic cells, of the type carried out by Kempner (*31*).

SUMMARY AND CONCLUSIONS

An extended study was made of the metabolism (Q values) and derived quotients of lymphoid, myeloid, monocytic, and atypical leukemias induced in young mice by methylcholanthrene skin painting, with or without X-irradiation. Lymph nodes, spleens, and livers were analyzed metabolically, examined histologically, and often tested for transmission. In general, the results obtained with the two stocks of mice employed, Rf and Rf/Ak, were similar, and no marked effects of sex, age, or X-irradiation were observed. The experimental results are summarized in a concluding table, together with all available data of other investigators on spontaneous leukemias studied to date.

The anaerobic glycolysis of lymph nodes and spleens of both Rf and Rf/Ak stocks with induced leukemias showed no definite metabolic distinctions (>20 percent) between control, preleukemic, or leukemic mice, irrespective of type or extent of infiltration in the leukemic group. This finding is in striking contrast with all reported results on spontaneous leukemias, where the lymph nodes display the increased anaerobic glycolysis indicative of, even if not specific for, malignant tumor tissue.

The anaerobic glycolysis of leukemic livers, however, showed, depending upon the extent of infiltration, a twofold to eightfold increase over controls, that took place simultaneously with histopathologic indication of onset of disease and successful transmission. All three types of leukemic analysis, metabolic, histopathologic, and transmission, concurred in indicating, after several months of painting, a sudden onset and rapid development involving but a few days. It could not be demonstrated from the few data obtained that the values for anaerobic glycolysis of livers from mice with transmitted leukemic or leukemoid (nontransmissible) infiltration were statistically different from values for primary leukemias. Since the anaerobic glycolysis of liver, in contrast with lymph node and spleen, showed a marked difference between the normal and leukemic, liver is an excellent organ for the study of possible preleukemic changes.

The respiratory quotients (R. Q.) of all tissues examined (Rf stock only) were well below unity, which is typical of both adult normal and malignant tissue, in contrast with growing normal tissue where the R. Q. ordinarily approaches unity. The oxidative metabolism (Q_{O_2} and Q_{CO_2}) of Rf lymph nodes and spleen showed a definite difference between normal and preleukemic organs on the one hand, and leukemic and leukemoid organs on the other, values for the latter groups being increased 25 to 75 percent. Unfortunately, no preleukemic oxidative values for liver were taken with either strain.

The Rf aerobic glycolysis values ($Q^{O_2}{}_A$) of preleukemic, leukemic, and leukemoid lymph nodes, spleens, and livers were definitely higher (50–100 percent) than normal values. The values for preleukemic nodes and spleen thus more nearly resembled those of leukemic tissue, with respect to aerobic glycolysis ($Q^{O_2}{}_A$), whereas they more nearly resembled normal tissue with respect to oxidative metabolism (Q_{O_2}, Q_{CO_2}). Both the relative and absolute increases in $Q^{O_2}{}_A$ of the leukemic and leukemoid livers were not so great as the corresponding increases in $Q^{N_2}{}_A$, owing to the usual operation of the Pasteur effect, and also to the aerobic oxidation by the liver cells of some of the lactic acid produced by the infiltrating leukemic cells.

Chemical lactic acid determinations (Q_{LA}) agreed in general with the manometric glycolysis values (Q_A) under anaerobic conditions, but there were interesting and expected differences under aerobic conditions, especially in liver. Thus, infiltrated leukemic and leukemoid livers produced lactic acid aerobically (Q_{LA} positive), but uninfiltrated normal livers consumed it (Q_{LA} negative), although manometrically all three groups produced total net acid (Q_A positive). Hence, determinations of the sign of Q_{LA} values of preleukemic livers, unfortunately not carried out in this study, would provide an excellent qualitative as well as quantitative test for the possible existence of a metabolically recognizable preleukemic state.

No evidence was obtained for the existence of any preleukemic metabolism different from normal metabolism, except possibly for a small increase in aerobic glycolysis that developed some weeks prior to the onset of leukemia. Whether this small increase represents a definite preleukemic state that is inevitably followed by leukemia, or some incidental result (e. g., hyperplasia) produced by the methylcholanthrene painting and X-irradiation treatments employed that bears no causal relationship to the incipient leukemia, remains to be determined.

In general, the metabolism of the induced leukemias, while qualitatively characteristic of malignancy, quantitatively probably

represents, together with certain chicken erythroleukoses, the lowest limit for any type of malignant tissue reported to date. The metabolism of most spontaneous mouse leukemias studied is only moderately malignant ($Q^{N_2}{}_A = 6-10\pm$). The less striking metabolism of these highly malignant blood cells, in contrast with the metabolism of most malignant-tumor aggregates, is possibly in some way related to their highly diffuse, circulating, systemic nature that introduces factors, as yet unrecognized, that reduce comparability.

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Action of 5,9,10-Trimethyl-1,2-Benzanthracene on the Skin of the Mouse

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INTRODUCTION

Badger and his coworkers (*1*) reported that the repeated application of dilute solutions of 5,9,10-trimethyl-1,2-benzanthracene in benzene to the skin of mice resulted in the appearance of melanomas in 3 animals of an initial number of 66. Two of these animals, which also had epitheliomas, were among a group of 26 that had been painted with a 0.06-percent solution of the compound. The last mouse in the group died on the one hundred and ninety-third day. The third animal with melanoma, which also had a papilloma, was 1 of 40 that had been painted with a 0.3-percent solution of the compound, and the last mouse of this group died on the one hundred and nineteenth day. Of the first group of 26 mice, 7 developed papillomas and 10 developed epitheliomas; while of the second group of 40 mice, 6 had papillomas and none had epitheliomas. The only information about these melanomas is the foregoing which is taken from a table in the original paper; there are no pathologic descriptions or photomicrographs, and the strain and color of the mice are not stated. Until this report, there had been no record of the production of a melanoma in a mouse by artificial means and only 2 reports in the case of any other species. Passey (*2*) applied a carcinogenic tar weekly to the skin of 12 dogs. Three tumors, all melanomas, developed after 6 to 7 years of tarring; of these, 1 tumor was undoubtedly and 1 was probably a malignant melanoma; the third was not examined microscopically but was said to be probably benign. In a later report (*3*) 2 more melanomas were obtained, and of the 5 melanomas, 3 were said to be malignant and 2 benign. It was stated, also, that spontaneous pigmented tumors, both malignant and benign, are not uncommon in dogs. Schürch (*4*) obtained a melanoma in a rabbit after 3.5 years of painting with a solution of 3,4-benzpyrene. Since the communication of Badger and coworkers, Belkin (*5*) reported a "melanotic sarcoma" in 1 of 800 Bagg albino mice that had been injected subcutaneously with a suspension of 10 mg. of 3,4-benzpyrene in glycerol; no other details were given regarding this tumor.

According to the literature, spontaneous malignant melanomas are also very rare in mice. Murray (*6*) mentions no melanomas in mice. Haaland (*7*) in a study of 353 primary tumors in 288 mice records only 1 melanoma, found in the connective tissue at the apex of a slit in the ear of a female black mouse. The tumor could not be transplanted. Heidenhain (*8*, *9*) records no melanomas in mice. Little and coworkers (*10*) in a study of nonepithelial tumor formation record 5 melanomas in 1,472 mice; all were found in breeding or virgin females of the C57 Black strain or crosses of this strain with the dba strain. Two were described as melanotic sarcomas. The following statement is made: "Melanomas, which are rare, are usually confined to the base of the tail and are deeply pigmented." In a later publication from

the same laboratory (*11*), it is stated that the melanomas are usually malignant. They "have occurred for the most part in our dba stock and the common site has been on or near the tail. There have been cases of melanoma of the eye, ear and the skin in general."

The present paper reports an attempt to induce melanomas in mice by artificial means, using inbred strains or their first-generation hybrids in which melanomas are known to appear spontaneously and a chemical agent which is reported to evoke this tumor in mice of unknown ancestry.

MATERIALS AND METHODS

A quantity of 5,9,10-trimethyl-1,2-benzanthracene (m. p. 127°–128° C.) was placed at our disposal by Dr. W. E. Bachmann, of the University of Michigan. According to Badger and coworkers (*1*), a 0.06-percent solution of this carcinogen produces a much higher incidence of cutaneous tumors (squamous-cell carcinoma and melanoma) than does a 0.3-percent solution. Consequently, the skin painting was carried out with the more dilute solution. On account of the instability of the substance, especially toward light, only 50 mg. was dissolved (in 83.3 cc. of thiophene-free benzene) at one time, and the solution was stored in a glass-stoppered flask in the dark when not in use. The painting was carried out twice weekly. For each application two strokes with a No. 5 camel's-hair brush were given along the midline of the back from the intersacral region to the base of the tail. The mice used were of the inbred strains dba, C57 Black, and I, and of the first-generation hybrids derived by reciprocal matings of the dba and C57 Black strains. All were derived from stocks maintained at the National Cancer Institute. The animals received a diet of Purina dog chow and an unlimited supply of water. In most instances an equal number of males and females, kept in separate glass jars, was used in each group. All mice were about 2 months old at the beginning of the experiment. The majority of the animals were permitted to live out their natural span of life. Some, however, were killed when they were already dying. The experiment was terminated nearly 10 months after the beginning of the painting. Except as noted later, all the mice were autopsied, and histologic studies were made of the painted area, hematopoietic tissues, and viscera.

RESULTS

Of the 124 mice used in this experiment some were discarded either because of early death, marked autolysis, or because they were victims of cannibalism. The results presented here are based on the 102 remaining animals (table 1). There was no apparent sex difference in the production of cutaneous lesions (table 2).

Within a week after the beginning of painting there was local loss of hair. Crust formation developed in 2 weeks, followed in some cases by ulceration. Some animals showed loss of appetite and marked decrease in weight.

Primary Tumors of the Painted Area

Papilloma and squamous-cell carcinoma

The incidence of papilloma in this experiment was somewhat lower and that of carcinoma higher than that reported by Badger and coworkers (*1*). The explanation is probably to be found in the longer duration of the present experiment. Carcinoma was usually preceded by the development of papilloma, and frequently the two lesions coexisted in the same mouse at autopsy. The first papillomas appeared at 39 days, in 2 strain I mice. The first carcinoma was found in a C57 Black mouse

TABLE 1.—*Incidence of tumors and pigmented foci of the skin and subcutaneous tissue in mice painted with 5, 9, 10-trimethyl-1,2-benzanthracene*

Strain	Number of mice	Sex		Maximum duration of painting	Papillomas only	Mice with—						
		Male	Female			Squamous-cell carcinoma			Subcutaneous sarcoma	Carcinosarcoma	Pigmented foci	
						Primary tumor	Metastasis					
							Lymph node	Lung				
	Number	*Number*	*Number*	*Days*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
dba	13	6	7	271	0	13	1	5	0	0	5	38
C57 Black	23	12	11	247	1	15	1	2	1	0	12	52
I	18	10	8	292	5	10	1	1	0	0	0	0
C57 Black × dba	30	15	15	258	4	23	2	3	6	2	28	93
dba × C57 Black	18	12	6	272	2	16	2	4	3	1	16	89
Total	102	55	47	.....	12	77	7	15	10	3	61	.....

coming to autopsy after 114 days of painting. Histologically, the squamous-cell carcinomas were similar to those produced by painting with other polycyclic hydrocarbons. Metastasis to the lymph node or lung occurred in 21 mice (table 1).

TABLE 2.—*Lack of sex difference in the incidence of skin lesions in mice following painting by 5, 9, 10-trimethyl-1, 2-benzanthracene*

Strain	Sex	Number of mice	Mice with—			
			Papillomas only	Squamous-cell carcinoma	Sarcoma	Pigmented foci
			No.	*No.*	*No.*	*No.*
dba	M	6	0	6	0	2
	F	7	0	7	0	3
C57 Black	M	12	1	7	1	5
	F	11	0	8	0	7
I	M	10	3	4	0	0
	F	8	2	6	0	0
C57 Black × dba	M	15	3	10	4	13
	F	15	1	13	4	15
dba × C57 Black	M	12	2	10	2	10
	F	6	0	6	2	6
Total	M	55	9	37	7	30
	F	47	3	40	6	31

Sarcoma

Subcutaneous fibrosarcoma beneath the painted area was noted in 13 mice. A much higher incidence was observed in the hybrids. No tumors were found in the dba or I strains and only 1 in the C57 Black strain. The first sarcoma was observed in a hybrid mouse coming to autopsy after 188 days of painting. Three of the connective-tissue sarcomas were mixed with squamous-cell carcinoma and yielded collision tumors. Of the 13 mice with sarcomas, all but 2 also had carcinomas, and except for the 3 collision tumors the 2 histologic types of tumor were separate and distinct. An unusual feature of these connective tissue tumors was that 5 of them were myxofibrosarcomas. No metastases were observed.

Hemangio-endothelioma

Four mice, one dba and the other three hybrids, showed hemangio-endothelioma in association with squamous-cell carcinoma. The gross and microscopic characteristics of these tumors were similar to those of the hemangio-endotheliomas in mice, occurring spontaneously or under experimental conditions, which have been reviewed and discussed in recent communications from this Institute (*12–14*).

PIGMENTED FOCI

Pigmented foci in the skin of the painted area were observed only in mice of the pigmented strains, dba and C57 Black, and in the hybrids, but not in the piebald

TABLE 3.—*The occurrence of pigmented foci of the skin at autopsy in mice painted with 5, 9, 10-trimethyl-1, 2-benzanthracene*

Strain	Number of mice	Mice with (+) and without (−) pigmented foci at autopsy						
		3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.	9 mos.
		Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
dba	13	0+	0+	0+	0+	0+	2+	5+
		0−	0−	0−	0−	1−	4−	8−
C57 Black	23	0+	1+	1+	5+	8+	12+	12+
		5−	5−	5−	5−	8−	10−	11−
C57 Black × dba	30	0+	0+	2+	6+	11+	26+	28+
		0−	1−	1−	2−	2−	2−	2−
dba × C57 Black	18	0+	2+	3+	6+	8+	14+	16+
		0−	0−	0−	0−	1−	1−	2−
Total	84	0+	3+	6+	17+	27+	54+	61+
		5−	6−	6−	7−	12−	17−	23−

I strain. The relative incidence of these foci in the hybrids was noticeably higher than in either of the parent strains. The rate of appearance of pigmented foci increased from the third month to the termination of the experiment, the greatest monthly increase being observed in animals coming to autopsy during the seventh month. Table 3 summarizes the occurrence of these lesions. The first lesion was noted in a C57 Black mouse which died 114 days after the beginning of painting. Since the mice were usually allowed to live out their natural life span, the majority, 52 in number, showed pigmented lesions associated in the same animal with malignant tumors such as squamous-cell carcinoma or fibrosarcoma. There were 9 other mice with pigmented foci in which no malignant tumor was present.

These foci occurred in the subcutaneous tissue of the painted area near the epidermal surface (fig. 1, *A*). They were as a rule sharply circumscribed but not encapsulated and varied in size from a small clump of a few cells to a nodule measuring 2 x 4 mm. (fig. 1, *B*). One or several were observed in histologic sections taken from a given painted area. Although no detailed study of the histogenesis of these lesions was made, it was possible in a number of cases to establish their connection with hyperplastic hair follicles. In virtually all the cases, these lesions did not appear to be neoplastic. The cells comprising them were round, oval, spindle-shaped, or sometimes stellate. The quantity of pigment in each cell was usually so great as to accentuate the cellular outline and largely to obscure the nucleus. The pigment did not give the prussian blue test for iron when applied to histologic sections. An attempt was made to dissolve the pigment out of the histologic sections in some instances in order to permit better observation of the cells containing it. The cytologic structures of the tissues so treated were not entirely satisfactory although a large part of the pigment was removed.

When caught in the path of an infiltrating tumor, the cells of the pigmented foci were dispersed and in part destroyed. Some were enmeshed in the connective tissue stroma of the neoplasm. In a few instances pigment was observed in nearby tumor cells composing the pearls of a squamous-cell carcinoma, possibly indicating phagocytosis. Other than this, a few of these pigmented foci, not sharply circumscribed and not intimately related to an infiltrating neoplasm, showed cells at the periphery which resembled neoplastic cells. These latter cells contained only a small amount of pigment. Whether

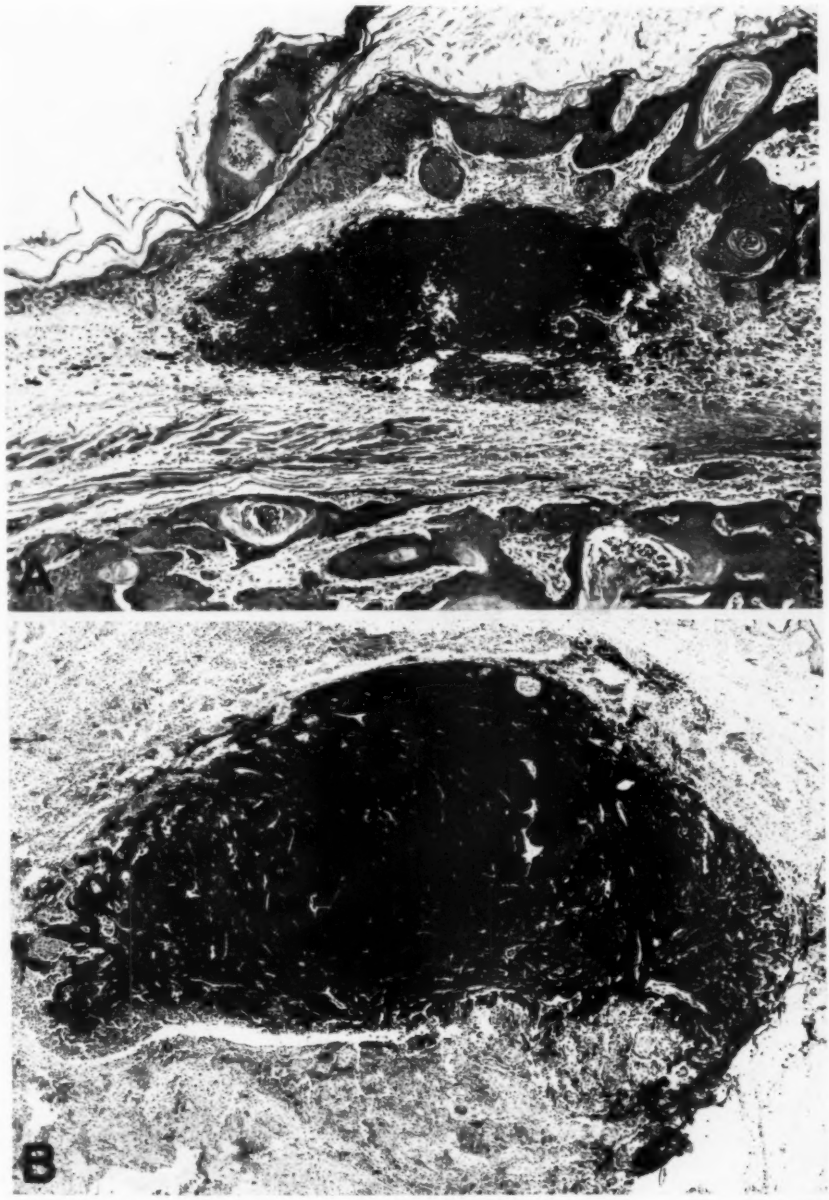

FIGURE 1.—*A*, Skin from painted area of a female C57 Black×dba hybrid mouse after 7.3 months of painting, showing pigmented focus. The lower area of the illustration shows infiltrating carcinoma. ×70; *B*, Large pigmented focus taken from beneath painted area, surrounded by necrotic squamous-cell carcinoma in a female dba×C57 Black hybrid mouse after 7.6 months of painting. ×28.

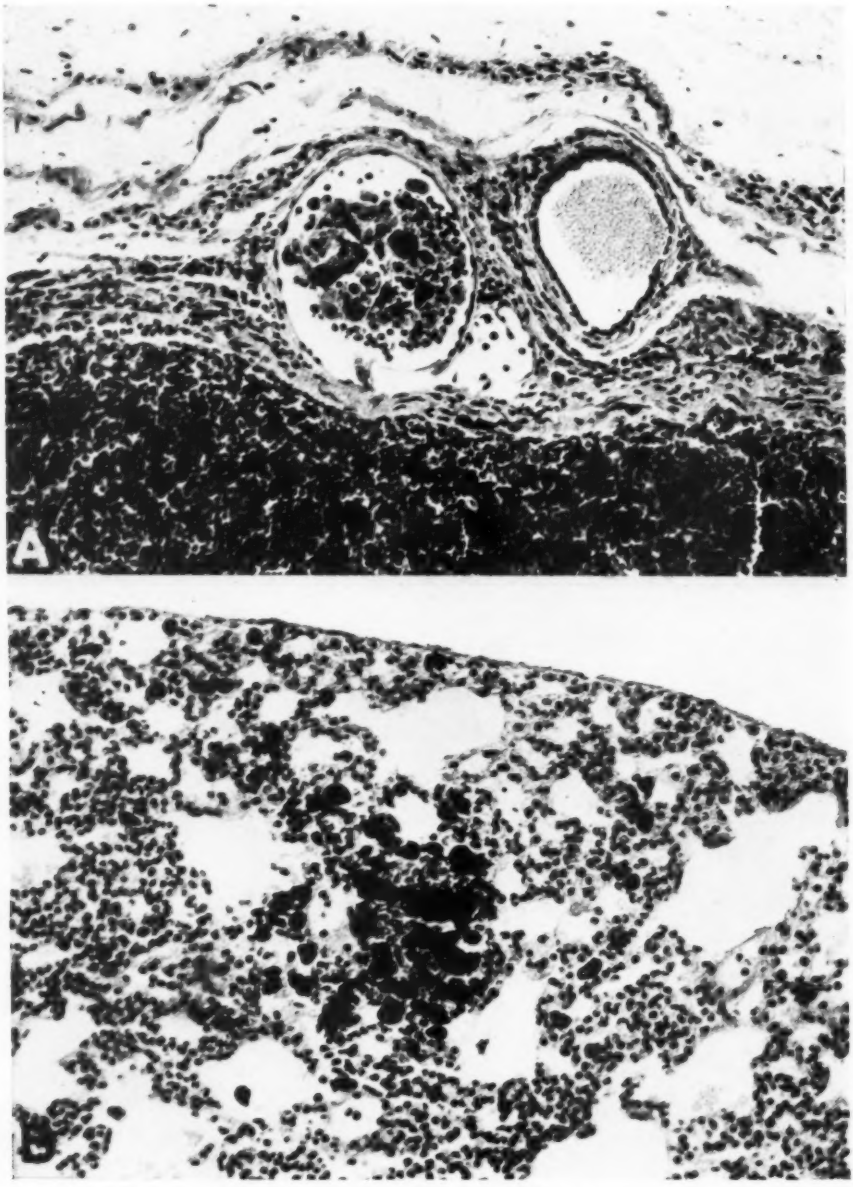

FIGURE 2.—*A*, Lymph node showing peripheral sinus containing pigmented cells. There are also numerous pigmented cells in the lymphoid tissue. × 187; *B*, Lung showing pigmented focus. × 210. Figures 1, *B*, and 2 are from the same animal.

they represented a neoplastic proliferation of the pigmented cells of the melanotic lesion or whether these new cells were arising from adjacent tissues could not be definitely determined. We have not considered the evidence sufficient for us to classify any of these lesions as neoplastic.

In several cases the lymph nodes contained diffusely scattered cells containing iron-negative brown pigment. In one lymph node (fig. 2, A) a peripheral sinus was distended with a large number of pigmented cells and with other cells some of which were suggestively neoplastic. In the mouse in which the lymph node (fig. 2, *B*) was seen, the lung contained a focus of pigmented cells identical with those noted in the skin. Locally, this mouse showed a squamous-cell carcinoma of the painted skin with widespread necrosis and ulceration (fig. 1, *B*). In view of this local destruction of tissue, it is thought possible that the pigmented foci in the lymph node and lung might represent the transport of degenerative and necrotic material from the painted area and not necessarily a metastatic tumor. This mouse had the largest pigmented focus in the skin, measuring 2 x 4 mm.

Miscellaneous Lesions

Ten animals, all hybrids, had small squamous papillomas of the forestomach. One possible explanation of these lesions is that they may have been induced by presumptive ingestion of the powerful carcinogen used in the painting.

Myeloid metaplasia of the spleen, liver, or lymph nodes was observed in 45 mice, distributed as follows: dba, 2; C57 Black, 6; I, 3; C57 Black × dba, 20; dba × C57 Black, 14. Lymphosarcoma or leukemia occurred in 3 C57 Black × dba and in 2 dba × C57 Black mice. In 2 cases these lesions were associated with a malignant cutaneous tumor, but in every case the animal also had pigmented foci in the painted area.

Amyloid of the spleen, liver, adrenal gland, or lymph nodes was observed in 50 mice distributed among the strains employed. Only 1, however, was seen in mice of the dba strain. This condition was usually associated with ulceration and infection of the painted area.

One hybrid mouse showed a cortical adenoma of the adrenal gland.

DISCUSSION

The experimental induction of papillomas and carcinomas of the skin in mice painted with a 0.06-percent solution of 5,9,10-trimethyl-1,2-benzanthracene in benzene (*1*) has been confirmed. The observations have been extended in the present experiments to include connective tissue tumors developing in the subcutaneous tissue of the painted area. These occurred either independently or as one of the components of mixed carcinosarcoma. Of particular note is the fact that 5 of the connective tissue tumors were myxofibrosarcomas. Squamous-cell carcinomatous metastases to the lymph node or lung occurred in 21 mice; none of the sarcomas metastasized. The results confirm the placing of this compound in the group of powerful carcinogens for mouse skin (*15*).

Bergmann and coworkers (*16*) described a local increase of pigmentation of the skin of CBA mice following the biweekly application of benzene alone. These investigators stated that at the end of 4 to 5 months the exposed skin at the site of painting became progressively darker, until finally the hairless area was jet black. Judging from their illustrations, the pigment seemed to be symmetrically distributed on both sides of the body, and in the histologic sections there was a linear

black pigmented strip in the corium with scattered pigmented cells sometimes reaching into the subcutaneous fatty tissue. We have not studied control mice painted with benzene alone. However, the pigmentation in our experimental mice differed from that just described in that the pigment occurred in variable-sized focal areas, haphazard in distribution, and without symmetrical arrangement.

Our results do not establish the ability of 5,9,10-trimethyl-1,2-benzanthracene to induce malignant melanomas. It is unfortunate that where a claim is made of the production of a rare type of experimental tumor (*1*), gross and microscopic descriptions as well as illustrations are not furnished. While, in our experiments, a high incidence of pigmented foci of the painted area was obtained, in no case could it be said with certainty, after microscopic examination, that any of these lesions were neoplastic. The pigmented foci are regarded, however, as evidence of proliferation of melanin-containing cells, and such foci may be somewhat analogous to the skin papillomas which precede the development of squamous-cell carcinoma of the painted skin. It is conceivable that if our animals had lived longer, some of these lesions might have developed into true malignant melanomas. It is also possible that some of the lesions observed might have exhibited sufficient autonomy on transplantation to have developed into malignant tumors.

Experiments were planned to test these and other points. We had also planned to vary the experimental conditions, strain of animal, dosage, length and frequency of painting, and to study changes in the painted skin treated with the Dopa technique in an attempt to provide a good experimental method for the production of this interesting tumor. The experiments reported here are preliminary and are being recorded because the present emergency renders uncertain the further prosecution of the work.

SUMMARY

One hundred and twenty-four mice of the inbred strains dba, C57 Black, and I, and of the first-generation hybrids derived by reciprocal matings of the dba and C57 Black strains, were painted on the skin of the back twice weekly with a 0.06-percent benzene solution of 5,9,10-trimethyl-1,2-benzanthracene. Of these, 102 were suitable for pathologic study.

The first papillomas appeared at 39 days in 2 strain I mice. Seventy-nine animals developed malignant tumors as follows: squamous-cell carcinoma, 77; fibrosarcoma, 10; mixed carcinosarcoma, 3. Squamous-cell carcinomatous metastases to the lymph node or lung occurred in 21 mice.

Pigmented foci in the painted area occurred in 61 mice. None of these lesions was considered to be a definite neoplasm. Their morphologic characteristics, nature, and significance are described and discussed.

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Creatine and Creatinine Content of Transplanted Hepatomas and of Normal and Regenerating Liver

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Creatine and its anhydride, creatinine, have long been known as components of many tissues and as products of excretion. According to the classic Folin theory, the production of these substances provides a measure of endogenous protein metabolism. Although this theory has been modified in part by the recent work of Schoenheimer (*1*) who has shown that creatine and creatinine formed by the animal may also come in part from exogenous precursors, it nevertheless remains fairly well established (*2*).

Creatine is formed in the tissues by the interaction of at least four compounds: choline, arginine, glycine, and methionine. The amidine group of arginine condenses with glycine to form guanidoacetic acid, which in turn is methylated by methionine or choline (*1*, *3*). This process may be carried out by different organs, for Borsook and Dubnoff (*4*) have shown that kidney tissue slices perform the synthesis of guanidoacetic acid and that liver tissue slices methylate the guanidoacetic acid. Muscle tissue, which contains by far the greatest proportion of creatine per gram of all the animal tissues, apparently obtains this substance by blood transport from the liver, since it is unable to synthesize creatine (*5*). The normal hepatic production of creatine, at least as it is understood at the present time, fulfills a dual role for (1) it provides muscle tissue with an indispensable component of the contractile process, and (2) it provides a method of removing end products of specific amino acid metabolism.

In order to see whether the capacity to produce creatine is retained when hepatic tissue becomes neoplastic, the content of creatine and creatinine was determined in the following tissues: transplanted hepatoma 31 in Osborne-Mendel rats (*6*), transplanted hepatoma 587 in A mice (*7*), transplanted hepatoma 7A/77 in A mice (*8*), the livers of rats and mice carrying these tumors, and the livers of normal animals. All the animals received a Purina dog chow diet. The mouse tumors were donated by Dr. Jesse E. Edwards.

EXPERIMENTAL PROCEDURE

The Jaffe reaction has been the basis of the classic method of determining creatine or creatinine in tissues. This method depends upon the production of a red color when creatinine reacts with an alkaline solution of picric acid. When creatine and creatinine are both present in a tissue, the latter is determined first by reaction with alkaline picrate; the tissue is then treated with hot acid under pressure and the creatine is transformed by cyclization and loss of water into creatinine. Reaction of the treated tissue with alkaline picrate gives the sum of preformed creatinine plus creatine, and subtraction of the former value from this sum gives, with the aid of a

correction factor, the amount of preformed creatine.

A vast literature on creatine and creatinine has accumulated on the basis of the Jaffe reaction. (See monograph by Hunter (*9*) and review by Beard (*10*).) A large part of this literature has been highly controversial in nature, largely because of the nonspecificity of the Jaffe reaction. It has long been known that alkaline picrate will react with many compounds to give a red color; and although a great deal of valuable and suggestive work has been accomplished with the aid of this reagent, its lack of specificity has prevented the placing of much of the data obtained on a firm and unequivocal basis.

In 1937 Dubos and Miller (*11*) introduced a specific method for the determination of creatine and creatinine. These investigators used strains of soil bacteria grown through several transfers in aqueous media which contained creatinine as the sole source of organic nitrogen and carbon. Such bacteria, according to Dubos and Miller, develop enzymes which attack only creatinine. The data on a large series of compounds related to creatinine by these investigators reveal a high degree of specificity for these "adapted" organisms (*11*, *12*, *13*). The method of Dubos and Miller consists in a determination of the chromogenic value of a tissue with alkaline picrate before and after incubation of the tissue with a sample of the specific bacteria. The difference in these values is a measure of the true creatine or creatinine of the tissue. Miller, Allinson, and Baker (*14*) and Baker and Miller (*15*) showed, in a series of studies of animal tissues, that whereas the chromogenic value of rat muscle tissue produced by alkaline picrate was almost wholly due to creatine and creatinine, the true creatine and creatinine content of rat liver formed only a fraction of the total chromogenic value for this tissue. For studies of hepatic tissue, therefore, the technique of Miller and Dubos is indispensable.

Soil from the grounds of this Institute was used as the source of the bacteria.[1] The soil was intimately mixed with a 0.5-percent solution of creatinine in distilled water to about half the water-binding capacity of the soil. After 48 hours of incubation at 36° C., an extract of the soil gave a negative test for creatinine. About 1 gm. of this soil was transferred to a wide-mouthed 1-l. Erlenmeyer flask and treated with 50 cc. of the salt solution plus phosphate buffer, yeast extract, and 25 mg. of creatinine as recommended by Miller, Allinson, and Baker (*14*).[2] After incubation for 48 hours at 36° C., the test for creatinine was negative. One cubic centimeter of this mixture was transferred to a fresh creatinine-containing solution, and this process of transfer repeated every few days. Up to the eighth transfer, the creatinine disappeared from the solution within 48 hours of incubation at 36°. At this point, the suspension was centrifuged at high speed, and the sedimented bacteria were taken up in a few cubic centimeters of ice-cold distilled water and used as the source of the creatinine-splitting enzyme.

The fresh hepatic tissues were prepared for determinations of creatine and creatinine according to the procedures of Miller, Allinson, and Baker (*14*). The necrotic areas of the transplanted hepatomas were previously removed. After several trials, sufficient tissue was employed in the tests to give a final creatine chromogen concentration of approximately 0.03 mg. per cubic centimeter of test solution. Aliquots of the latter were incubated at 36° C. with a suspension of the bacteria for the period of time necessary for the same volume of suspension to destroy 10 times the amount of creatinine

[1] *The soil must not be used soon after a rainfall.*
[2] *Use of Bethesda tap water was unsatisfactory.*

TABLE 1.—*The content of creatine and creatinine in normal and neoplastic hepatic tissues* [1]

Tissue	Number of animals	Total creatine chromogen	Residual creatine chromogen	True creatine	Total creatinine chromogen	Residual creatinine chromogen	True creatinine
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	
Transplanted hepatoma 31 (thirty-fifth transplant)	6	32	22	10	1.5	1.5	.....
Livers of rats bearing transplanted hepatoma 31	6	33	21	12	1.6	1.5	.....
Normal rat liver	6	33	22	11	1.4	1.4	.....
Regenerating rat liver (48 hours after operation)	2	34	24	10	1.5	1.5	.....
Transplanted hepatoma 587 in A mice (fourth transplant)	4	33	28	5	1.2	1.2	.....
Transplanted hepatoma 7A/77 in A mice (fourth transplant)	4	32	26	6	1.2	1.2	.....
Livers of A mice bearing transplanted hepatoma 587	4	36	30	6	1.4	1.4	.....
Livers of A mice bearing transplanted hepatoma 7A/77	4	35	30	5	1.4	1.4	.....
Normal A mice liver	4	34	28	6	1.2	1.1	.....

[1] Values for creatine and creatinine are given in milligrams per 100 gm. of tissue and on the basis of wet weight of the tissue.

in distilled water. About 6 hours was required by 0.5 cc. of the suspension to destroy 2.4 mg. of creatinine in 10 cc. of water at 36°. During the period aliquots of the test solution were incubated with added water in place of the suspension. At the end of the 6-hour period, the chromogenic values with alkaline picrate were determined for the treated and untreated test solutions (*14*). The difference between these values gave the content of true creatine or creatinine. Longer periods of incubation, up to 48 hours, did not cause a significant increase in this difference. The results (table 1) represent the average values with a variation of ± 5 percent for a number of animals.

The data in table 1 reveal the striking fact that the values of total chromogen are practically the same for all the normal and neoplastic hepatic tissues of both rats and mice. The true creatine values of all the rat hepatic tissues are very nearly the same, as are the true creatine values of the mouse hepatic tissues, but the values for the rat are about double those for the mouse. Differences due to the different water content of the various tissues (*16*) are within the range of variation of these substances. The value for true creatinine is vanishingly small in all the tissues and lies within the range of variation. It would be unjustified to express it in any terms at present, and it has, therefore, been omitted from the table. The value for true creatine in the rat tissues is about 30 percent and in the mouse tissues about 15 percent of the total chromogen. The nature of the residual chromogen is unknown. Obviously, it is formed as a result of the acid treatment of the tissue. According to Baker and Miller (*15*), rat liver has by far the greatest amount of this non-creatine chromogen. In descending order appear lung, spleen, kidney, pancreas, ovary, intestinal muscle, brain, testis, heart muscle, diaphragm, and gastrocnemius muscle. The relatively small amount of non-creatinine chromogen in the native, untreated, hepatic tissues is practically the same for all and is only a small fraction of the chromogen that appears after acid treatment of the tissue.

The absolute values of the total creatine and creatinine chromogen in normal rat liver are very nearly the same as those reported by Baker and Miller (*15*) and by Chanutin (*17*), and the value for true creatine is somewhat greater than that

reported by the former investigators (*15*). Nakahara, Kishi, and Fujiwara (*18*) reported that the total creatine and creatinine chromogen content of a transplanted rat hepatoma was the same as that of a normal rat liver, but their data for normal liver differ widely from those of Miller, Chanutin, and the author. The Japanese workers reported values of 5 mg. creatine chromogen percent and of 5 mg. creatinine chromogen percent, the former being almost seven times lower, and the latter almost five times higher than the corresponding values reported by Miller, Chanutin, and the author. The reason for this discrepancy is not apparent, but it may be due to either differences in rat strains or to differences in the experimental technique used.

The apparent fact that the creatine and creatinine content of the transplanted hepatomas is the same as that of normal liver suggests that hepatic tissue retains its specific creatine metabolism, presumably with the concomitant transmethylating and transamidinating systems, when the normal tissue becomes neoplastic. The retention of a specific chemical system in this tissue when it changes to a hepatoma is similar to that found in the case of the amylase system (*19*) and is in marked contrast with the loss or diminution of glycogen (*6*) and riboflavin (*16*) and of several enzyme systems (*19*, *20*). The animals used for these studies had a diet presumably adequate in creatine and its precursors. It would be of great interest to see whether the tumor creatine would be affected by a diet inadequate in these precursors.

SUMMARY

The creatine and creatinine content of the following normal and tumorous hepatic tissues was determined by the specific enzymatic method of Miller and Dubos: transplanted rat hepatoma 31, normal and regenerating rat liver, transplanted hepatomas 587 and 7A/77 in A mice, normal mouse liver, and the livers of hepatoma-bearing rats and mice.

The content of total creatine chromogen in all the rat and mouse hepatic tissues was practically the same, as was also the content of total creatinine chromogen. The content of true creatine was the same for all the rat hepatic tissues, whether normal or neoplastic; the content of true creatine was also the same for the mouse hepatic tissues, but this value was nearly half that found for the rat tissues. The true creatinine content of all the rat and mouse hepatic tissue was vanishingly small.

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Incubation of Citrulline and Ammonia With Normal and Neoplastic Hepatic Tissues

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INTRODUCTION

The greater part of the urea formed by the normal animal is produced in the liver. The mechanism for the hepatic synthesis of urea has been described by Krebs and Henseleit (*1*) as follows:

(1) Ornithine + Ammonia + Carbon Dioxide → Citrulline
(2) Citrulline + Ammonia → Arginine
(3) Arginine → Ornithine + Urea

The process is evidently cyclic in nature. It represents the method whereby the liver removes the otherwise toxic ammonia, derived from the oxidative deamination of amino acids, and converts it into the innocuous urea. Reactions (1) and (2), which are due to as yet unidentified synthetic enzyme systems, are demonstrated *in vitro* only in the presence of liver tissue slices. Liver extracts are ineffective. Reaction (3) is produced by the hydrolytic enzyme arginase, and this reaction occurs equally well in the presence of either slices or extract. Although the liver contains by far the greater proportion of arginase in the animal body, other tissues contain small amounts of this enzyme. The production of urea by the latter tissues is most probably owing to the action of the arginase on arginine either brought to the tissues by the blood or produced in the tissues by the breakdown of proteins.

The arginase activity of hepatomas in rats and in mice is very much lower than that of the normal liver of these species (*2, 3*). In order to see whether the synthetic enzymatic reactions are also altered when the liver tissue becomes neoplastic, slices of transplanted hepatomas were incubated with mixtures of citrulline and ammonia (reaction (2)).

EXPERIMENTAL PROCEDURE

The tissues used for this study included the transplanted hepatoma 31 in Osborne-Mendel rats (*4*) and normal rat livers. The necrotic areas were carefully removed from the tumors and discarded.

l-(+) Citrulline was prepared from *l*-(+) arginine by the method of Gornall and Hunter (*5*). The yield of purified citrulline was 5 gm. from 30 gm. of arginine monohydrochloride. The stock solution of citrulline was prepared by dissolving 1 gm. in 25 cc. of Ringer's solution (*1*). The source of added ammonia was a 1.2-percent solution of ammonium chloride in Ringer's solution.

The incubation fluid for the rat tissue slices was rat serum from Osborne-Mendel rats. For the rat hepatoma slices, the serum of normal and of hepatoma-bearing rats was separately employed, but no difference in the results was observed.

The tissue slices were placed in each of two flasks containing 2.0 cc. of serum and weighed to the nearest milligram. To one flask 0.25 cc. of ammonium chloride solution plus 0.25 cc. of Ringer's solution were added; the other flask was treated with

0.25 cc. of ammonium chloride plus 0.25 cc. of the citrulline solution. The wet weight of the tissue slices in each flask was about 75 mg. Six rat hepatomas and six rat livers were studied separately.

The flasks containing the slices were placed in an incubator at 37° C. and shaken for 2 hours in an atmosphere of 95 percent oxygen and 5 percent carbon dioxide. At the end of this time, the flasks were centrifuged, and 1 cc. of the supernatant was removed from each flask. Each sample of the supernatant was treated with 3 cc. of water and 1 cc. of saturated potassium carbonate, and the liberated ammonia was blown over into a sulfuric acid trap and estimated. The estimation of ammonia in these samples indicated how much of this substance had been consumed by the reaction. If it combined with citrulline to form arginine (reaction 2) and the latter was hydrolyzed to form urea and ornithine (reaction 3), the amount of urea nitrogen formed should be just twice that of the ammonia nitrogen consumed. The other half of the urea nitrogen came from citrulline. Consequently, a second cubic centimeter of the supernatant was removed from each incubation flask, treated with 2 cc. of water and 20 mg. of jack bean powder containing urease, and incubated at 37° for 30 minutes. At the end of this period the samples were treated with alkali and aerated, and the liberated ammonia was determined. Blank determinations of the amount of ammonia and urea present in the tissues and in the serum were, of course, taken into account in the calculations. No reaction was observed between citrulline and ammonia in the absence of the tissue slices. There was likewise no effect whatever of either the tissue slices or of the jack bean powder on citrulline alone.

The dry weight of the tissues was calculated from the wet weight by independent determinations of the moisture content in separate samples of each tissue.

The concentration of preformed urea nitrogen present in the serum was 0.20 mg. per cubic centimeter.[1] Since the serum was diluted from 2 to 2.5 cc. with Ringer's solution for the present studies, the con-

[1] *The content of preformed ammonia in the serum and of preformed ammonia and urea in the tissue slices was negligible in quantity.*

TABLE 1.—*The incubation of citrulline and ammonia* [1]

Tissue	Dry weight of tissue	Residual ammonia N in—		Consumed ammonia N	Total ammonia N after action of urease in—		Urea N
		Slices plus ammonia	Slices plus ammonia and citrulline		Slices plus ammonia	Slices plus ammonia and citrulline	
	Milligrams per cubic centimeter	*Milligrams per cubic centimeter*	*Milligrams per cubic centimeter*	*Milligrams per cubic centimeter*	*Milligrams per cubic centimeter*	*Milligrams per cubic centimeter*	*Milligrams per cubic centimeter*
Normal rat liver	37	0.17	0.10	0.07	0.35	0.42	0.16
Do	32	.14	.08	.06	.31	.34	.10
Do	30	.14	.07	.07	.32	.34	.11
Do	41	.16	.07	.09	.31	.38	.15
Do	40	.16	.09	.07	.31	.37	.12
Do	35	.11	.02	.09	.27	.36	.18
Transplanted rat hepatoma 31	50	.26	.26	.00	.42	.42	.00
Do	43	.23	.23	.00	.38	.38	.00
Do	43	.21	.21	.00	.38	.38	.00
Do	47	.17	.17	.00	.33	.33	.00
Do	40	.22	.22	.00	.37	.37	.00
Do	35	.20	.20	.00	.37	.37	.00

[1] Before incubation with tissue slices, each cubic centimeter of sample contained 0.31 mg. ammonia N. In addition, one sample contained 4 mg. citrulline. The incubation period was 2 hours. One cubic centimeter of this digest was tested for ammonia content. Another cubic centimeter was incubated 30 minutes with urease, and the ammonia content was then determined. Serum urea N content was 0.16 mg. per cubic centimeter.

centration of urea nitrogen was 0.16 mg. per cubic centimeter. The amount of urea nitrogen formed by the reaction was obtained by subtracting the sum of this quantity and the residual ammonia nitrogen from the total ammonia nitrogen present after urease treatment. Thus, in table 1, the calculated values for urea nitrogen (column 8) are obtained by adding 0.16 to each figure in column 4 and subtracting the sum from the appropriate figure in column 7.

The data (table 1) reveal that under the conditions whereby a relatively rapid rate of urea synthesis occurs in normal liver tissue slices, no evidence for such synthesis occurs when slices of the transplanted rat hepatoma are used. For the normal liver tissues the urea nitrogen calculated (column 8, table 1) was double the ammonia nitrogen consumed (column 5) in only two of the six cases studied; in the other cases the ratio was somewhat less. This is most probably due to the fact that all three reactions formulated in the Introduction proceeded simultaneously, and since ornithine reacts with two moles of ammonia whereas citrulline reacts with only one, more ammonia was consumed than would be accounted for by the reaction with citrulline alone. In the hepatoma tissue slices, no ammonia was consumed by the citrulline, and this is confirmed by the identity of the figures in columns 6 and 7, table 1. The use of the serum of tumor-bearing rats or the addition of 20 mg. of sodium lactate did not alter the findings.

Of further interest is the apparent loss of ammonia which occurred when ammonium chloride alone was incubated with the tissue slices in serum suspension. Before incubation each cubic centimeter of the latter fluid contained 0.31 mg. of ammonia nitrogen. After the incubation each cubic centimeter of the liver-slices suspension contained 0.11–0.17 mg.; each cubic centimeter of the tumor-slices suspension contained 0.17–0.26 mg. of ammonia nitrogen (column 3). There was evidently a consumption of ammonia by the tissue slices themselves. The original quantity of ammonium chloride added to serum alone or to a liver or tumor extract prepared in serum was almost quantitatively recovered (0.30 mg. N). The property of taking up ammonia is thus characteristic only of the intact surviving cells. It is possible that the ammonia thus consumed combines with the products derived from the sugar of the serum. This assumption is based upon the finding of Warburg, Posener, and Negelein (*6*) that the ammonia developed by tissue sections in sugar-free Ringer's solution disappears if sugar is added to the medium. In any event, it is apparent that the amount of such consumed ammonia is distinctly greater in the liver than in the hepatoma slices.

DISCUSSION

The lack of evidence for the presence of the urea synthesizing system in the transplanted hepatoma is consistent with other findings in the comparison of enzymatic activity of hepatomas with normal liver tissues (*3, 7*). Many, but not all, of the specific functional properties of normal liver are considerably decreased when this tissue becomes neoplastic. This relation of tumor to the tissue of origin appears to be fairly general. Thus, the following enzymes which are highly active in normal tissue are either decreased in activity or absent in the following tumors: pepsin and phosphatase in gastric adenocarcinoma (*8*), phosphatase in intestinal adenocarconoma (*8*), and depolymerase in lymphomas (*9*). Indeed, the quantitative activity for each enzyme in a number of tumors of various origin, is. with few exceptions, more nearly

alike than the activity of the same enzyme when compared in the normal tissues of origin of these tumors. It would be interesting to consider the approach of tumors, regardless of their origin, to a more or less common enzyme level denominator. This point will be considered elsewhere. It need only be emphasized here that in general whenever a functional normal tissue is characterized by the possession of a highly active enzyme system, it suffers the loss of part or all of this system when the tissue becomes neoplastic.

The finding that the tissue slices take up ammonia in the presence of whole serum suggests a synthetic reaction involving the ammonia and the metabolic products of the serum and tissue carbohydrate. The greater consumption of ammonia by the liver than by the tumor slices might be explained by the fact that the sugar content of the former tissue is greater than the latter. The hepatoma contains little if any glycogen (*4*). On the other hand, the aerobic glycolysis of the tumor is considerably greater in the hepatoma than in normal liver (*10*). The intermediate stages in the conversion of glucose into lactic acid in either tumor or liver tissue are not clear at the present time. Whether ammonia combines with the same intermediate product in both tissues, such as pyruvic acid to form alanine, or at different stages in each tissue to form quite different products, remains a subject for future consideration.

SUMMARY

Citrulline and ammonia were incubated in an atmosphere of O_2–CO_2 with a serum suspension of rat liver and of transplanted-rat-hepatoma-tissue slices. Under conditions whereby urea is relatively rapidly synthesized by the normal liver slices, no evidence for such synthesis occurred when slices of the hepatoma were used. The relation of this finding to other results obtained on comparing the enzymatic activity of tumors and the normal tissues of origin is discussed.

Both liver and hepatoma slices in serum took up ammonia in the absence of citrulline, the former tissue to a greater extent than the latter. This effect was not produced by extracts of the tissues in serum and appeared to be dependent upon the presence of intact cells.

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Induction of the Carbon Tetrachloride Hepatoma in Strain L Mice[1]

By JESSE E. EDWARDS, *research fellow*, W. E. HESTON, *geneticist*, and ALBERT J. DALTON, *cytologist, National Cancer Institute, National Institute of Health, United States Public Health Service*

In earlier communications on the induction of the carbon tetrachloride hepatoma in mice, it was reported that this tumor has been obtained frequently in mice of the C3H strain (*1*), which has a high incidence of spontaneous hepatoma, as well as in those of strains A, Y, and C, which develop relatively few spontaneous hepatomas (*2*). Our results with carbon tetrachloride hepatoma induction in mice of strain L, another of the low-spontaneous-hepatoma strains, are presented in this paper.

MATERIALS AND METHODS

The mice used in this experiment were of the inbred strain L (C57 leaden) colony maintained at the National Cancer Institute. The colony was started with animals obtained from Dr. A. M. Cloudman, at the Roscoe B. Jackson Memorial Laboratory, in 1938. The leaden mutation (*ln*), discovered by Murray (*3*), arose in the C57 Brown strain, which in turn was derived from the progenitors of the C57 Black strain. Strain L is a highly inbred strain, the animals of this experiment being of the thirty-first generation of inbreeding by brother-to-sister matings since the leaden mutation arose.

The incidence of spontaneous hepatomas in this strain is extremely low. Of 51 virgin untreated mice (23 males and 28 females), which were autopsied at 15 months of age, 1 male presented a hepatoma. In a second group of 50 virgin mice (22 males and 28 females) autopsied at 18 months of age, none showed this neoplasm. In a group of 51 breeders (24 males and 27 females), which were autopsied at 12 months or older, 1 male showed a hepatoma. This animal was autopsied at the age of 23 months. The presence of 2 spontaneous hepatomas in 152 mice gave an incidence of 1.3 percent for this tumor.

As in the earlier experiments, carbon tetrachloride of a high degree of purity was administered in olive oil by stomach tube usually three, but occasionally two times weekly. A 40-percent solution of carbon tetrachloride was used and was administered in a volume of 0.1 cc. at each treatment, each mouse thus receiving 0.04 cc. per treatment. The mice were fed Purina dog chow pellets and were allowed a free supply of tap water.

The figures reported herein are taken from 73 mice which had received a total of 46 feedings by stomach tube during a period of 4 months. There were 19 females and 54 males, ranging in age from 2.5 to 7.5 months, at the beginning of the experiment. The animals were killed and autopsied 3 to 3.5 months following the last treatment, with the exception of 1 mouse, which was autopsied 6 weeks after the forty-sixth administration. The mice varied from 8.5 to 14 months of age at autopsy. The final diagnoses were made

[1] *Referred to as C57 leaden in Mouse Genetic News No. 1 (1941), of which G. D. Snell is the editor. This strain is also referred to as strain M by certain workers.*

on the basis of microscopic examination, except for a small number of animals with obvious tumors which were saved for gross mounting as museum specimens.

RESULTS

Of the 73 mice in this series, 34, or 46.6 percent, showed hepatomas. The results are summarized in table 1. It will be observed that the incidence of hepatoma in the younger group, in which the mice ranged between 2.5 and 3.5 months of age at the start of the experiment, was essentially similar to that for the older group, in which the animals were 3.5 months of age or older when the experiment was begun. In only 1 subgroup, which was composed of females belonging to the older group, was the incidence of hepatomas induced considerably below the general average. The number of mice in this subgroup was too small for the figures to be statistically significant, however.

TABLE 1.—*Incidence of hepatomas in strain L mice*

Sex of mice	Age at start of experiment	Number of mice	Mice with hepatoma	Incidence of hepatomas
	Months		*Number*	*Percent*
Male	2.5–3.5	15	7	46.7
Female	2.5–3.5	8	3	37.5
Total or average		23	10	43.5
Male	3.5–7.5	39	21	53.8
Female	3.5–7.5	11	3	27.3
Total or average		50	24	48.0
Both age groups: Total or average		73	34	46.6

The appearance of the hepatomas was fundamentally similar to the carbon tetrachloride hepatoma described for other strains of mice (*2*). The tumors were usually multiple, appearing as gray or grayish-yellow bulging nodules ranging from 2 to 15 mm. in diameter. Microscopically, the tumors were not encapsulated, yet not invasive (fig. 1, *A*).[2] The adjacent hepatic tissue was compressed by their rounded borders. The tumor cell showed a close resemblance to the hepatic parenchymal cell with prominent cytoplasmic membranes and round, vesicular nuclei (fig. 1, *B*). The cytoplasm often showed a faintly basophilic cast and was either granular or vacuolated (fig. 2, *A*). In a number of tumors, numerous spherical or ovoid cytoplasmic inclusions were present which were either red or faintly green in color with the haematoxylin and eosin strain (fig. 2, *B*). Often these inclusions were so numerous that they replaced most of the tumor cell cytoplasm. Mitotic figures varied in number. The patterns of the Golgi apparatus and mitochondria characteristic of carbon tetrachloride hepatoma induced in other strains (*4*) were also found in these tumors. The Golgi apparatus was in the form of a well-developed juxtanuclear network, while the mitochondria were slender twisting filaments characteristic of induced hepatoma. The tumor cells were arranged in cords which alternated with endothelial-lined sinusoids (fig. 3). At times evidence of this cordlike arrangement was accentuated by the presence of wide blood channels. At other times the sinusoids were narrow, even collapsed, resulting in an apparently solid sheetlike arrangement of the tumor cells. In these cases, however, the cordlike arrangement was actually maintained.

In a small number of tumors there were foci of lymphocytic infiltration which at times were associated with identifiable remnants of bile ducts. In one animal, a healed pyelonephritis occurred, with amyloidosis of the liver and spleen, and although the liver was heavily and diffusely infiltrated with amyloid, a hepatoma

[2] *All the photomicrographs inserted were prepared from sections of tissue stained with haematoxylin and eosin.*

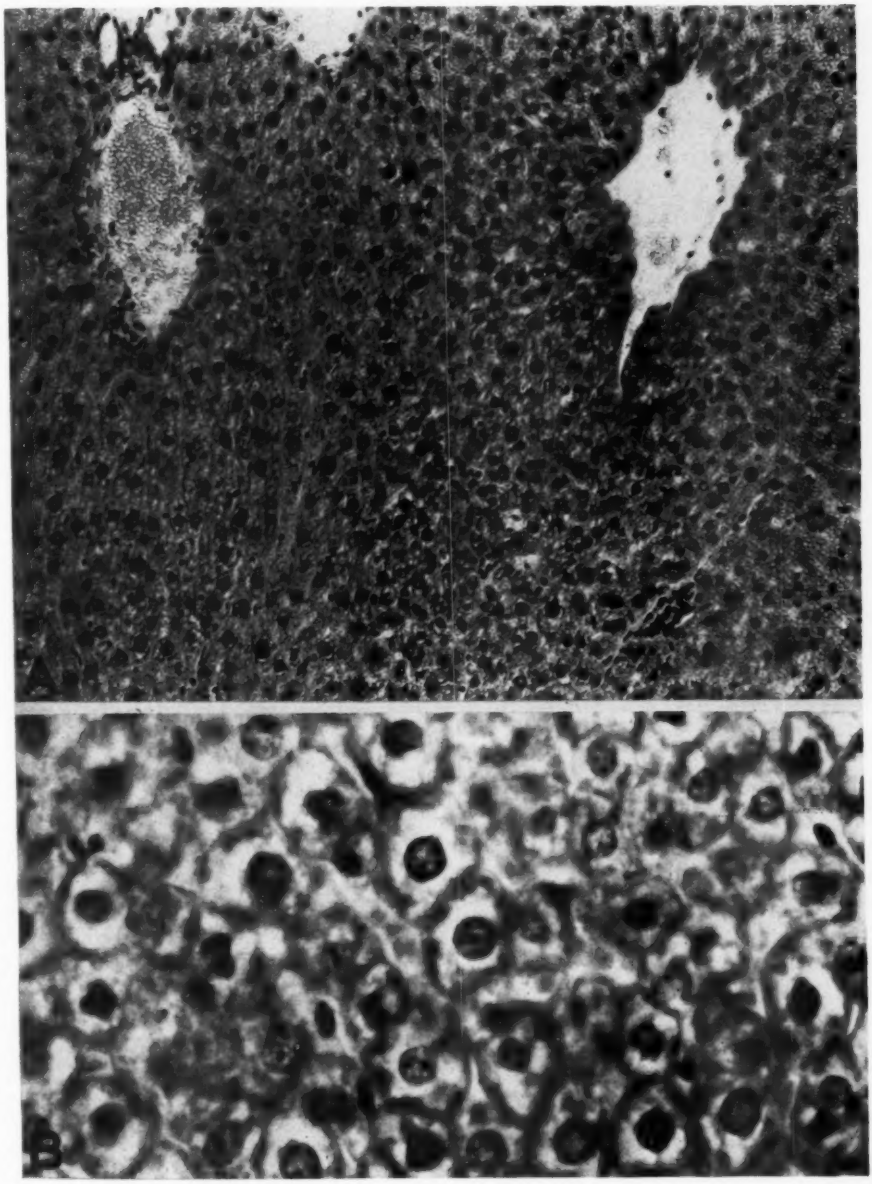

FIGURE 1.—Carbon tetrachloride hepatoma. *A*, Hepatoma and adjacent hepatic tissue. Hepatoma in right half of photomicrograph. Note lack of encapsulation and liverlike appearance of tumor. ×210; *B*, Liverlike cells with prominent cytoplasmic membranes. Moderate vacuolization of cells. ×465.

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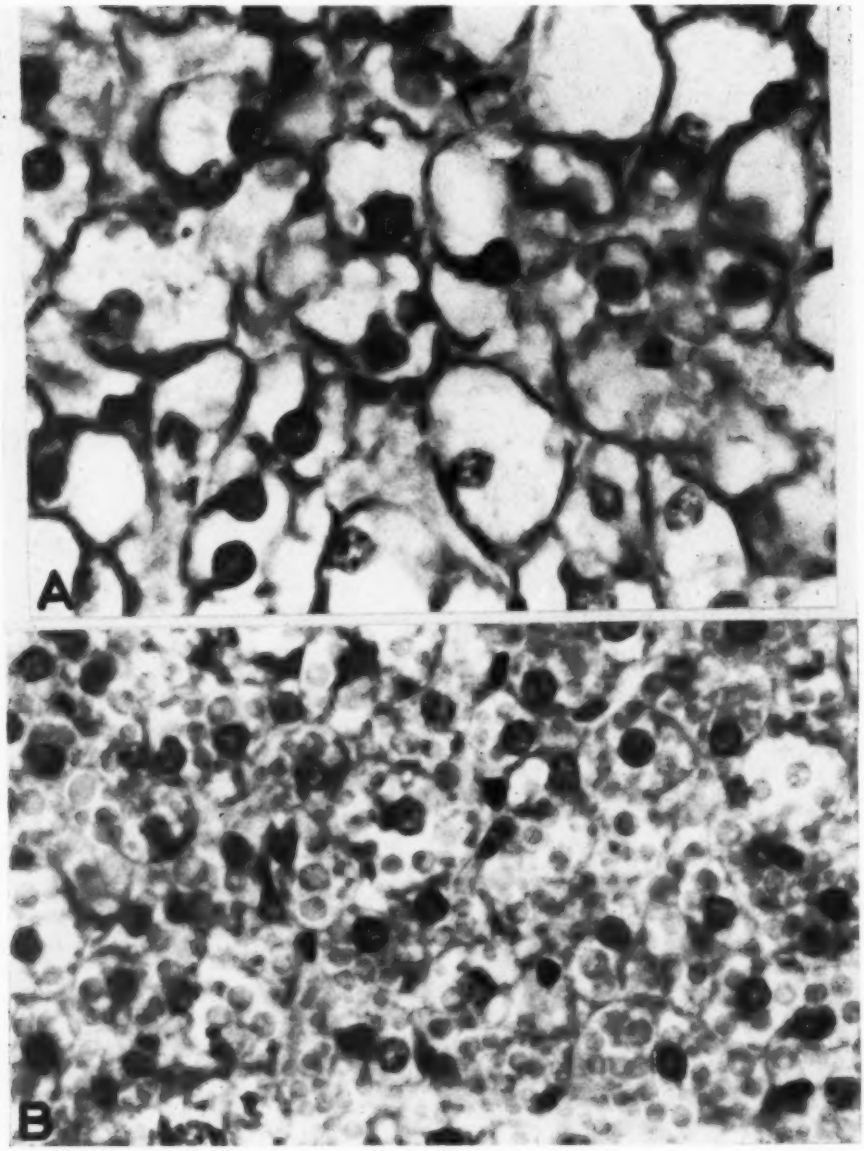

FIGURE 2.—Carbon tetrachloride hepatoma. *A*, Highly vacuolated and swollen cells. Compare the size of the tumor cells with those in figures 1, *B* and 2, *B*, which are of approximately equal magnification. ×475; *B*, Numerous intracellular spherical inclusions. ×465.

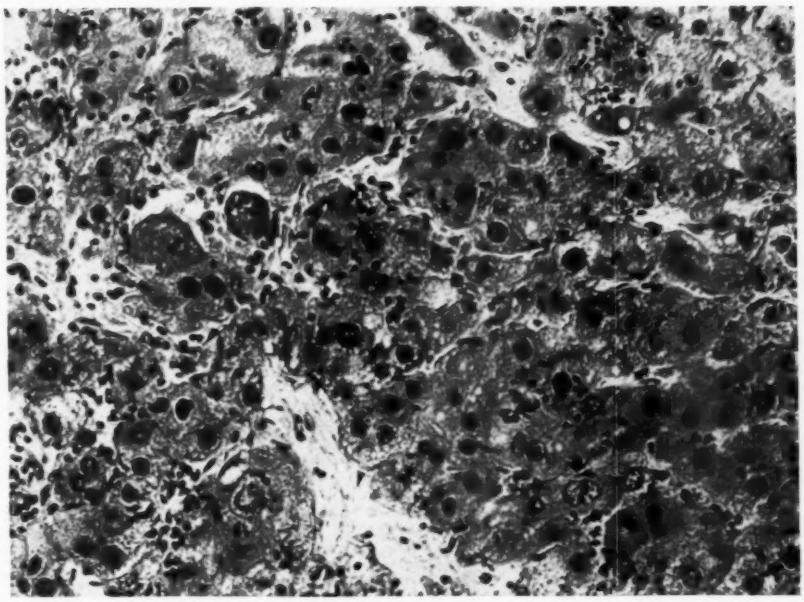

FIGURE 3.—Cordlike arrangement of carbon tetrachloride hepatoma. ×255.

present showed only small amounts of this substance distributed in a focal manner. None of the tumors observed appeared to invade blood vessels, and there were no metastases.

SUMMARY

Carbon tetrachloride in olive oil was administered to strain L mice three or two times weekly by stomach tube in hepatotoxic doses of 0.04 cc. carbon tetrachloride per treatment, for 46 treatments.

Of 73 male and female mice, which varied from 2 to 7.5 months in age at the start of the experiment and from 8.5 to 14 months at autopsy, 34, or 46.6 percent, developed hepatomas. The induced tumors were similar to the carbon tetrachloride hepatoma of other strains already described.

The incidence of spontaneous hepatomas in a group of 152 virgin and breeding male and female strain L mice from 12 to 23 months of age was 1.3 percent.

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Relationship Between the Lethal Yellow (A^y) Gene of the Mouse and Susceptibility to Induced Pulmonary Tumors

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It has been shown that susceptibility to induced pulmonary tumors is associated with known inherited unit characters of the mouse. A previous communication (*1*) reported that backcross mice homozygous for the linked genes, shaker-2 and waved-2 (*sh–2sh–2 w–2w–2*), were less susceptible than those heterozygous for the normal alleles (*Sh–2sh–2 W–2w–2*). Furthermore, backcross mice with flexed tails (*ff*) were less susceptible than those with normal tails (*Ff*).

The data presented in this paper show that the lethal yellow (A^y) gene has an effect on susceptibility to induced pulmonary tumors in that its presence increases susceptibility.

DESCRIPTION OF THE A^y GENE

The lethal yellow gene was one of the early known genes of the house mouse, having been studied by Cuénot (*2*) as early as 1905. It has since been found to be a member of the agouti allelic series and has proved to be particularly interesting in that it has a number of manifestations. The effect of the gene is lethal when it occurs in the homozygous (A^yA^y) condition, the embryos dying in the early stages of development (*3, 4, 5*). Mice heterozygous for the gene, however, are viable. They have a bright yellow coat, and they also show a tendency to become obese (*6*).

STRAINS USED AND PROCEDURE

Strain Y males were used to introduce the A^y gene. These males were from Dr. Andervont's strain Y colony maintained at the National Cancer Institute. The stock came originally from the Roscoe B. Jackson Memorial Laboratory in 1936. Brother-sister inbred generations total 17 since 1936, and the stock had been inbred prior to that time. Thus, for practical purposes at least, it is safe to assume that the strain is homozygous for all factors with the exception of the A^y gene and probably closely linked genes. Yellow coat color has been selected, but the strain has remained heterozygous for the character due to the lethal effect of the homozygous condition. The stock carries the non-agouti allele.

Data from Andervont's colony show that the incidence of spontaneous pulmonary tumors in strain Y mice is between 10 and 20 percent (*7*), and Shimkin's work (*8*) with induced pulmonary tumors classified the strain as medium in degree of susceptibility to the induced tumors.

Strain A, which has been extensively used in pulmonary-tumor work, was selected as the high-pulmonary-tumor strain. The animals used here were of the strain A colony maintained at the National Cancer Institute. This colony was derived from mice obtained from the Roscoe B. Jackson Memorial Laboratory in 1938. The strain can be considered homozygous

for practically all factors since brother-sister inbred generations now total over 60. The degree of susceptibility to pulmonary tumors is very high (*9, 10, 11*). Incidences ranging from 70 to 90 percent have been reported for spontaneous pulmonary tumors, and the strain is also extremely susceptible to induced pulmonary tumors. From previous work it has been reported that of strain A mice injected intravenously with 0.5 mg. 1,2,5,6-dibenzanthracene in horse serum, 100 percent had developed pulmonary tumors as early as 6 weeks after receiving the injection.

Strain A females, which were albinos but which also carried non-agouti and brown, thus having the genotype *aa bb cc*, were mated to the yellow strain Y males whose genotype was $A^{y}a$ *bb CC*. This mating produced an F_1 hybrid generation, of which one could expect approximately 50 percent to be yellow ($A^{y}a$ *bb Cc*) and the other 50 percent to be brown (*aa bb Cc*). Because of the inbreeding of the parent strains, one could assume that this F_1 generation was isogenic except for these genes, lethal yellow and non-agouti, and probably also the closely linked genes.

Ninety-one of these F_1 hybrids were produced and tested to determine whether or not the yellow mice differed from their brown litter mates in susceptibility to induced pulmonary tumors. At 8 weeks of age each animal was injected intravenously with 0.5 mg. 20-methylcholanthrene dispersed in 0.5 cc. normal horse serum.[1] At 20 weeks of age (12 weeks after the injection), the animals were killed, the presence of pulmonary tumors was noted, and the nodules appearing on the surface of the lungs were counted. Counting was done with the dissecting microscope, a procedure that has been followed extensively in induced-pulmonary-tumor work by Lynch (*12*) as well as by the author (*10*).

All the test hybrids were maintained under identical conditions. They were kept in wooden cages, eight animals to the cage, and were retained as virgins throughout the experiment but were not segregated as to color. They were given a constant supply of tap water and Purina dog chow.

The test animals were weighed at 8 weeks of age when they were injected and again 12 weeks later at the time they were killed.

RESULTS

The results clearly show that the yellow hybrids were more susceptible to induced pulmonary tumors than were their brown litter mates (table 1). At 12 weeks following injection, all the mice had developed pulmonary tumors, but the difference in susceptibility was evident when the number of nodules per individual was considered. The yellow mice had developed more nodules per individual than had the brown, the mean number for the yellow being 9.78 while that for the brown was only 4.18. The difference of 5.6 nodules is statistically significant ($t=6.554$; $P<0.01$).

The weights of the animals were in accord with those published for previous work with A^{y} gene (*12, 15*) (table 2). At both weighings the yellow mice were heavier than their brown siblings, and also the yellow mice showed more gain in weight over the 12-week interval between weighings than did the brown. Table 2 shows that the yellow mice gained as much as 15.75 gm. while the brown mice gained only 5.16 gm.

At the time of injection the yellow mice averaged 5.36 gm. more in weight than did the brown, yet the mice of both color groups were injected with the same amount of carcinogen. Thus, it should be noted that in spite of what one might expect on

[1] *Prepared by Dr. Egon Lorenz, of the National Cancer Institute.*

TABLE 1. *Development of pulmonary tumors in AYF_1 hybrids 12 weeks after the intravenous injection of 0.5 mg. 20-methylcholanthrene*

Genotype	Number of mice	Mice showing given number of tumors per indivíual																			Mean number of nodules	Standard deviation
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	25		
$A^y a$ (yellow)	41	–	1	–	1	5	4	3	4	6	3	2	1	2	2	3	1	1	1	1	9.78	4.59
$a\ a$ (brown)	50	10	9	7	8	4	3	2	2	1	1	–	–	2	–	1	–	–	–	–	4.18	3.32

the basis of dosage per unit body weight, the yellow mice developed more nodules than did the brown.

TABLE 2.—*Average weight of mice of the two different genotypes resulting from crossing strain A to strain Y*

Genotype	Number of mice	Average weight at—	
		8 weeks	20 weeks
		Grams	*Grams*
$A^y a$ (yellow)	41	28.90	44.65
$a\ a$ (brown)	50	23.54	28.70

DISCUSSION

From the data of the experiment it is evident that the chromosome bearing the A^y gene influenced susceptibility to induced pulmonary tumors in that its presence rendered the animals more susceptible to the induced neoplasm. The F_1 hybrid test animals produced by mating the strain A females to the strain Y males could be considered isogenic except for this 1 pair of chromosomes. Of the 91 hybrids, 41 received the A^y gene and 50 were homozygous for the non-agouti gene. Since these mice were all reared under the same conditions and were all subjected to the same treatment, the increase in susceptibility of the yellow mice over that of the brown must have been due to the A^y gene or to some other factor segregating with the A^y gene.

Further analysis shows that the increase in susceptibility was probably due to the A^y gene per se. If another factor had been involved, it would have had to be linked with the A^y gene but not carried on the homologous chromosome in the strain Y mice. This seems unlikely. The strain has remained heterozygous in respect to the A^y gene since homozygous A^yA^y mice are nonviable and since yellow coat color has been selected. However, with the crossing over that would have occurred in the chromosome, one could reasonably expect that the extensive inbreeding to which the strain has been subjected would probably have led to homozygosity for many of the other factors on the chromosome, expecially those not closely linked with the agouti locus. Thus, this would tend to localize the factor influencing susceptibility at least to the vicinity of the agouti locus.

Additional evidence on this point can be cited from a previous test which failed to demonstrate linkage between susceptibility to induced pulmonary tumors and the agouti locus (*14*). In this case strain A was outcrossed to strain W which carried the light-bellied agouti allele. If in strain Y there is a susceptibility factor closely linked with the A^y gene, such a factor is not found in strain W.

Thus, while it is realized that the evidence does not constitute absolute proof that the effect demonstrated here was due to the A^y gene and not to a closely linked factor for which strain Y was heterozygous, it seems highly probable that the increased susceptibility of the yellow mice over that of the brown was due to the A^y gene.

It is interesting that a factor introduced

by the more resistant parent strain should have a positive effect toward susceptibility. Such a situation can well be expected, however, when one is dealing with a quantitative character inherited on a multiple-factor basis as susceptibility to pulmonary tumors has been shown to be (*10*). Castle (*13*) and Law (*15*) found that certain coat color genes increased body size of backcross hybrids irrespective of whether the coat color genes in question came from the larger or smaller parent strain. A second somewhat analogous situation is that reported by Russell (*16*) for an inherited tumor in *Drosophila melanogaster*. She was able to demonstrate that the effect of chromosome III of the susceptible strain was that of decreasing the tumor incidence although chromosome *II* carried an essential gene or genes and chromosome I carried a gene or genes increasing incidence.

After finding evidence that the A^y gene increases susceptibility to induced pulmonary tumors, one immediately begins to search for a physiologic interpretation of the manner in which this result may be manifested. Little (*17*) showed that yellow mice tended to develop mammary gland tumors earlier than did their non-yellow litter mates, although the total incidence was less for the yellow than for the non-yellow mice. As a probable explanation of these results, he pointed out that the yellow mice passed through their reproductive period earlier than did the non-yellow, a fact which not only could explain the earlier appearance of the tumors but also the lower total percentage. It does not seem probable that this condition would influence susceptibility to pulmonary tumors if only the sex hormones were involved. If the condition were that of a more generalized aging process, however, pulmonary tumor development might be affected.

A more probable explanation of the results for induced pulmonary tumors reported here is that there may be a relationship between the increased susceptibility of the yellow mice and their increased body weight. Danforth (*6*) showed that yellow mice tend to become obese, and Castle (*13*) later demonstrated that the yellow mice were not only fatter than their non-yellow litter mates but were also larger in certain body measurements, such as body length and tail length. In the course of this experiment, the yellow mice gained over 10 gm. more than did their brown litter mates, the brown mice being of normal size. Thus, the overweight yellow mice were more susceptible than were the brown of normal weight.

There is an increasing amount of evidence in the literature which indicates that in certain cases at least there may be a positive correlation between body weight and susceptibility to tumor development. Various workers in the field of human cancer are of the opinion that there is an increased liability to cancer in persons who are overweight (*18*). One must bear in mind, however, the dangers of attempting to correlate rather generalized conclusions from human material with specific results from experimental animals. Nevertheless, there may later prove to be some grounds for comparisons between these observations on human beings and those of this experiment.

Observations which may be more closely related to those presented here are the ones which Tannenbaum (*19*, *20*) made in his experiments with mice. He showed that mice fed a restricted diet, which retarded growth, were less susceptible to a number of types of tumors, primary pulmonary tumors included, than were the normal controls. In another experiment mice fed a high-fat diet tended to be more

susceptible to certain types of tumors than were those fed a control diet. In this latter case, increase in susceptibility was not found for pulmonary tumors. However, the difference in weight between the mice fed the high-fat diet and the controls was not nearly so great as the difference between the yellow and brown mice of the present experiment.

Loeb and his associates (*21*) have concluded from some of their recent work that greater body weight of mice is one of the factors that favor the development of mammary carcinoma. Within various susceptible inbred strains, they have found a positive correlation between the frequency of mammary carcinoma and the average body weight.

Thus, while the shortcomings of these comparisons are fully realized, there is a suggestion that the physiologic processes which are associated with the A^y gene and which result with the increase in body weight may also be responsible for the increase in susceptibility to induced pulmonary tumors. Furthermore, these processes may be the same as those which cause an increased incidence of tumors in mice fed certain diets resulting in increased body weight and also those responsible for increased cancer incidence in overweight human beings. The suggestion, however, can serve only as a working hypothesis until further experimentation has been done.

SUMMARY

Yellow F_1 hybrid mice resulting from mating strain A females to strain Y males were more susceptible to induced pulmonary tumors than were their brown litter mates. The evidence presented indicates that this increase in susceptibility was due to the A^y gene per se.

It is suggested that the physiologic processes which are associated with the A^y gene and which lead to increased body weight of the yellow mice over that of the brown litter mates may also lead to the increased susceptibility to induced pulmonary tumors of the yellow mice.

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Technique Suitable for Quantitative Studies on the Mammary Tumor Inciter of Mice

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Following Bittner's (*1*) observation that an agent or agents in mothers' milk is essential for the development of mammary tumors in inbred strains of mice, a series of investigations was undertaken to lay the foundation for quantitative determinations which may be necessary for the elucidation of the nature of the agent. It was found (*2*) that the first milk is not essential for the production of tumors and that the agent is present in the milk of high-mammary-tumor-strain females throughout the lactation period. The next investigation (*3*) showed that mice 7 to 14 days old from a high-mammary-cancer strain were susceptible to the agent. These results established a source of the agent as well as a suitable test animal for the determination of its presence.

The experiments reported at this time are a continuation of these studies, and their primary object was to ascertain whether susceptible mice will develop mammary tumors when measured amounts of milk obtained from high-mammary-tumor-strain females are forcibly fed or injected subcutaneously, and if so, whether the occurrence of tumors is correlated with the amount of milk administered. In the first experiment mice 7 to 10 days old were fed milk or given subcutaneous injections of milk, lactating mammary gland, or spleen. In the second experiment subcutaneous injections of milk were given to nursing mice and mice 30 to 57 days old, while in the third experiment adult mice from 3.5 to 4 months of age were forcibly fed milk. The purpose of the second and third experiments was to determine the relative susceptibility of mice of different ages to the activity of the agent. The fourth experiment established the use of hybrid mice as satisfactory test animals.

MATERIALS AND METHODS

Mice of strains C3H, C57 Black, and I were used. All mice were born and raised in this laboratory. Strain C3H animals (*4*), descendants of mice originally procured from the Roscoe B. Jackson Memorial Laboratory, were employed as the high-mammary-tumor strain. Strain C57 Black mice were used as the low-mammary-cancer strain. These were descendants of litters obtained from Bagg (*5*), of the Memorial Hospital, New York, and from Murray (*6*) of the Springville, N. Y., Biological Station. Strain I mice were descendants of animals originally obtained from L. C. Strong, of the Yale University Medical School.

Strain C3H mice and I female by C3H male hybrids were used as experimental animals. The C57 Blacks were used only as foster mothers for the C3H mice.

Pregnant strain C3H mice were examined twice daily, at 9 a. m. and 4 p. m. except on Sundays or holidays when they were examined once daily. As the young

were born they were transferred to C57 Black foster mothers. Thus the maximum time the C3H young spent with their motners was from 7 to 20 hours. When the foster-nursed C3H young were used experimentally at 7 to 10 days of age, or after weaning, part of each litter was kept as untreated controls. These litter-mate controls are essential because C3H mice may develop mammary cancer (*2*) if they remain with their mothers for only a few hours after birth.

The mice were weaned when 1 month of age, bred, and permitted to give birth to one litter which was killed within 24 hours after birth, since strain C3H mice bearing one litter develop mammary tumors at an earlier age (*4*) than do virgin females. All mice were kept under similar environmental conditions and were maintained on an unlimited supply of Purina dog chow pellets and tap water.

All milk and tissues administered to the fostered C3H mice were obtained from lactating strain C3H females. Two methods were used to procure milk: In the first, C3H females nursing their first or second litter were removed from the young at 4 p. m. during the tenth to fifteenth day of lactation. The following day they were killed by cervical dislocation and the mammary glands removed by blunt dissection. The glands were put into a tissue mincer, and the milk was squeezed out by pressure. The milk was strained through fine gauze, or centrifuged at low speed for 5 minutes to remove larger tissue particles, and then used for feeding or injection. This method gave an average of 2 to 3 cc. of milk from each female. This milk is referred to as mincing-machine milk. In the second method, strain C3H females nursing their first, second, or third litter were removed from their young during the tenth to seventeenth day of lactation, and on the following the day milk was obtained by means of an automatic milking device designed by Kahler (*7*), of the National Cancer Institute. This method produced an average of 0.5 cc. of milk from each mouse. The milk, which was not strained or centrifuged, is referred to as milking-machine milk.

The milk obtained by both methods was kept at 4° C. when not in use and was always used the day it was procured. Forced feeding was accomplished by using a 1-cc. syringe and a blunted, curved, 22-gage needle. The maximum amount fed to the mice 7 to 10 days old at one time was 0.2 cc. Thus, mice receiving 0.2 cc. were fed once, those receiving 0.4 cc. were fed during the morning and afternoon of the same day, while those fed 0.8 cc. were fed morning and afternoon of two successive days. The young were always removed from their C57 Black foster mother 1 hour before the forced feeding.[1]

Lactating mammary tissue and splenic tissue were taken from lactating strain C3H females that were removed from their litters overnight. In most instances half the mammary tissue was used to obtain milk which was fed to young mice. This was done to test for the presence of the agent in the milk of mice supplying mammary and splenic tissues. The other half of the mammary gland was cut into fine pieces with scissors, and 0.1 cc. was injected subcutaneously into the right axilla. The spleen was cut into five equal portions and each portion injected subcutaneously by means of a trocar. Aseptic precautions were observed when preparing milk, mammary glands, or spleen for subcutaneous administration.

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[1] *In later experiments this procedure was modified successfully. The young were removed from their foster mothers for 1 to 2 hours, fed a dose of 0.2 cc., and 1 hour later fed the second 0.2 cc. The feeding of 0.8 cc. was terminated.*

EXPERIMENTAL PROCEDURE

Before the experiments were undertaken, an investigation was completed which ruled out the possibility that contact alone is responsible for the occurrence of mammary cancer in mice. In all previous work in this laboratory there was no evidence that bodily contact without ingestion of milk produced mammary cancer. Fostered and nonfostered litter-mate C3H mice were kept in the same cage after weaning, and tumors did not occur in the fostered animals. The possibility remained that contact with their mother during the nursing period may offer an opportunity for the agent to infect the young. To settle this question, the nipples of pregnant C3H mice were seared a day or two before they cast their litters. When the litters were born a lactating C57 Black mouse was placed in the cage. This procedure kept the young C3H mice in close contact with their mother while they obtained only strain C57 Black milk. Of 20 strain C3H mice raised under these conditions none developed a mammary gland tumor although they lived to an average age of 20 months. Apparently the agent responsible for the occurrence of mammary cancer in strain C3H mice is not contagious through body contact.

Experiment 1

The first experiment was begun during October 1940 and included only fostered C3H mice 7 to 10 days old as experimental animals. Some received measured amounts of mincing-machine milk by the oral or subcutaneous route; others received 0.2 cc. of milking-machine milk orally; while others were given subcutaneous injections of lactating mammary gland or spleen. The experiment is summarized in table 1 in which the animals are grouped according to the amount of substance administered or the route of administration, and each group consists of the experimental animals and their litter-mate controls. The feeding of 0.1 to 0.8 cc. of mincing-machine milk or 0.2 cc. of milking-machine milk produced tumors in from 77 to 100 percent of the experimental animals. This showed that tumors can be evoked in susceptible mice 7 to 10 days old if the milk is forcibly fed and served as a basis for an investigation (*8*) in which strain C3H milk was fractionated by means of an ultracentrifuge, and the fractions were fed to foster-nursed C3H young in the manner described herein.

The results of the subcutaneous injection of milk or lactating mammary gland show that the agent is capable of producing tumors if administered by this route. Following the injection of lactating mammary gland 6 mice developed tumors before they were 7 months of age, suggesting that mammary tissue may be rich in the cancer-inciting agent. This led to the assumption that mammary tumors in mice contain a large amount of the agent, and consequent studies (*8*) in which extracts of tumors were fed to mice 7 to 14 days old showed that the tumors are an excellent source of the agent.

The subcutaneous injection of splenic tissue induced tumor in 52 percent of the experimental animals. This indicates that the agent is in the spleen and is, therefore, widely disseminated throughout the body of lactating strain C3H females.

Tumors occurred in the litter-mate controls of all groups, the incidence ranging from 14 to 42 percent. These tumors were probably due to small amounts of C3H milk ingested by the young before they were given to C57 Black foster mothers. An analysis of the data by excluding all those experimental mice whose sister controls developed tumor gave results almost identical with those presented in table 1.

TABLE 1.—*Experiment 1. Occurrence of mammary gland tumor following the administration of strain C3H milk, lactating mammary gland, or splenic tissue to 7-to-10-day-old fostered strain C3H female mice*

[Numerator = number of mice developing tumor
Denominator = number of mice dying without tumor]

Group	Number of mice	Substance administered	Route of administration	Dose	Age (in months)													Incidence of mammary tumor	Average tumor age	Average age of mice dying without tumor
					5	6	7	8	9	10	11	12	13	14	15	16	17	Total			
1:				*Cc.*															*Per cent*	*Months*	*Months*
Experimental	28	Mincing-machine milk	Oral	0.1		3/0		4/0	1/0	2/0	2/0	4/0	4/0	2/0	2/1	1/0	1/1	26/2	93	11.2	16.0
Control	24					2/0	1/0	1/0		1/0	2/0	1/0		2/0	0/2	0/5	0/7	10/14	42	9.9	16.4
2:																					
Experimental	53	Mincing-machine milk	Oral	.2	1/0	1/0	2/1	14/0	4/0	7/0	6/0	2/0	1/1	5/0	1/2	0/1	0/4	44/9	83	9.8	14.9
Control	36					2/0	1/1	1/0	1/0	1/0	3/0			2/1	1/10	0/5	0/7	12/24	33	10.2	15.4
3:																					
Experimental	26	Mincing-machine milk	Oral	.4	1/0	3/0	1/0	4/0	2/0	5/0	2/0	4/0	1/0	2/0		1/0		26/0	100	9.9	
Control	22							2/0	1/0	1/0				0/1	0/2	0/12	0/3	4/18	18	8.7	15.9
4:																					
Experimental	24	Mincing-machine milk	Oral	.8		1/0	3/0	4/0	5/0	2/0	3/0	3/0	2/0	1/0				24/0	100	9.7	
Control	19								2/0		1/0			0/4	1/2	0/9		4/15	21	11.0	15.3
5:																					
Experimental	35	Milking-machine milk	Oral	.2	1/0			1/0	3/0	7/0	5/0	6/0	2/0	2/0	0/8			27/8	77	10.8	15.0
Control	19				1/0			1/1	2/0	2/0		1/0	1/0	0/2	0/8			8/11	42	9.5	14.2
6:																					
Experimental	7	Mincing-machine milk	Subcutaneous	.1				2/0	2/0			1/0			1/0		0/1	6/1	86	10.2	17.0
Control	4											1/0				0/1	0/2	1/3	25	12.0	16.7
7:																					
Experimental	26	Mincing-machine milk	Subcutaneous	.2				4/0	3/1		5/0	1/0	2/0	2/0	1/0	1/1	0/5	19/7	73	11.1	15.7
Control	14												2/0	0/2		0/4	0/6	2/12	14	13.0	16.2
8:																					
Experimental	17	Lactating mammary gland.	Subcutaneous		3/0	3/0		2/0		4/0					1/0		0/4	13/4	76	8.0	17.0
Control	10							1/0	1/0					0/1	0/1	0/4	0/2	2/8	20	8.5	15.9
9:																					
Experimental	23	Spleen	Subcutaneous				1/0	2/0	1/0	2/0	3/0			1/0	2/5	0/6		12/11	52	10.7	15.5
Control	17					1/0		1/0		1/0	1/0			1/0	0/1	0/11		5/12	29	9.8	15.9

Experiments 2 and 3

In experiments 2 and 3 an effort was made to ascertain the influence of age upon susceptibility to the milk agent. The results are combined in table 2 in which groups 1 and 2 are from experiment 2, and groups 3 and 4 from experiment 3. Litters in which control mice developed tumors are omitted. Mincing-machine milk was used in both experiments. In experiment 2 the milk was injected subcutaneously into fostered C3H mice ranging in age from 12 to 57 days. The injection of 0.15 to 0.25 cc. of milk into mice 12 to 23 days of age produced tumors in 11 of 16 animals, while the injection of 0.5 cc. into mice 30 to 57 days old induced tumors in only 2 of 14 animals.

In experiment 3 fostered C3H mice 3.5 to 4 months of age were used. They were removed from food at 3 p. m. and at noon the following day were fed 0.5 cc. or 1.0 cc. of milk by stomach tube. Two hours later they were given the standard diet. One of twelve mice fed 1.0 cc. and 2 of 12 fed 0.5 cc. developed tumors.

Experiment 4

In view of the occurrence of tumors in the control mice of experiment 1 in which fostered C3H mice were used, it was considered advisable to search for a more suitable test animal. The ideal test mouse should be as susceptible to the milk agent as those of strain C3H but should not receive any of the agent from its mother. Previous work (*9, 10*) indicated that mice derived by outcrossing strains I and C3H were very susceptible to the development of mammary cancer, for, when strain C3H females were mated to strain I males, the resultant female offspring showed an incidence of 96 percent mammary tumors at an average age of 8.5 months if used as breeders, and an incidence of 90

Table 2.—*Experiments 2 and 3. The occurrence of mammary gland tumors following the administration of strain C3H milk to fostered strain C3H female mice*

[Numerator = number of mice developing tumor
Denominator = number of mice dying without tumor]

Group	Number of mice	Age of mice at time of administration	Route of administration	Dose	Age (in months)																	Total	Incidence of mammary tumors	Average tumor age	Average age of mice dying without tumor
					6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22				
		Days		*Cc.*																			*Percent*	*Months*	*Months*
1: Experimental	16	12–23	Subcutaneous	0.15–0.25	1/0	1/0		1/0	4/0	0/1				2/1		1/0	0/1		1/1	0/1		11/5	69	11.7	17.0
Control	10	12–23					0/1						0/1		0/2	0/1	0/3	0/1		0/1		0/10	0		16.5
2: Experimental	14	30–57	Subcutaneous	.5						0/1	0/1			0/1	2/1		0/1	0/2	0/3		0/2	2/12	14	16.0	18.0
Control	15	30–57						0/2		0/1			0/1		0/1	0/3	0/1	0/1	0/2	0/2	0/1	0/15	0		16.7
		Months																							
3: Experimental	12	3.5–4	Oral	1.0									1/0	0/1						0/10		1/11	8	14.0	20.0
Control	9	3.5–4																		0/9		0/9	0		21.0
4: Experimental	12	3.5–4	Oral	.5						0/1				0/1	0/1	1/1		0/1		0/5		2/10	17	12.5	18.3
Control	11	3.5–4						1/0						0/1	0/2					0/8		0/11	0		19.5

percent tumors at an average age of 11.4 months if kept as virgins. It had also been noted in an earlier experiment (*11*) that mammary tumors did not occur in a small group of female hybrids obtained by mating strain I females to strain C3H males. These observations suggested that hybrids derived from a strain I mother and strain C3H father may be better test animals than fostered C3H mice, and an experiment was performed to test this possibility.

During December 1940 strain I females were mated to strain C3H males. The young were born during January and February 1941; and as they were born some females from each litter were transferred to strain C3H foster mothers for a period of 24 hours, after which they were returned to their strain I mothers for the remainder of the nursing period. The other females in each litter remained with their strain I mother during the entire nursing period. This gave two groups of animals, one consisting of I × C3H hybrid females exposed to the milk agent in strain C3H milk for 24 hours during the first 48 hours of life, the other consisting of litter-mate controls which did not obtain any strain C3H milk. The mice were weaned when 1 month of age and marked individually. When from 3 to 4 months of age each gave birth to a litter which was killed within 24 hours after birth. The animals were kept under observation for the occurrence of mammary tumor until they were 15 months of age.

The results of the experiment were clear-cut. There were 49 mice in each group. Of those nursed by strain C3H foster mothers for 24 hours, 42, or 85 percent, developed mammary tumors at an average age of 8.3 months. All 7 survivors of the group that did not develop tumor lived to 15 months of age. Of those nursed by their strain I mothers only, none developed a mammary tumor and all lived to be 15 months of age.

DISCUSSION

The results of experiment 1 show that fostered C3H mice 7 to 10 days of age will develop mammary cancer if forcibly fed 0.1 to 0.8 cc. of strain C3H milk. The 93-percent incidence of tumors in mice fed 0.1 cc. suggests that this amount may be considerably above the minimum effective dose. For this reason the final results of the experiment do not reveal any significant correlation between the amount of milk administered orally and the occurrence of tumors, but an analysis of the findings 14 months after beginning the study (*8*) showed a relationship between dose and tumor response. Lower doses should be used to ascertain the dose-response relationship.

It is also shown that milk obtained by a milking device produced tumors when administered orally. This milk does not contain tissue particles or juices which are present in the mincing-machine milk.

The occurrence of mammary cancers in susceptible mice following oral administration of the mammary tumor inciter is in accord with the findings of other investigators. Bittner fed milk (*12*), lyophilized mammary-tumor tissue (*13*), and extracts of mammary-tumor tissue (*14*). Visscher, Green, and Bittner (*15*) fed fractions obtained from lactating mammary glands.

The production of mammary tumors by subcutaneous injection of milk is also in harmony with the results of others who used other materials. Bittner (*16*) used pieces of splenic, thymic, and lactating mammary tissue. Woolley, Law, and Little (*17*) injected whole blood, while Visscher, Green, and Bittner (*15*) injected

fractions procured from lactating mammary glands. Bittner (*14*) also injected mice intraperitoneally with filtrates of lactating mammary glands or glycerolated tumor extracts. These observations show that the agent is distributed widely through the body of the mouse and can evoke neoplasms in susceptible mice when administered parenterally.

The outcome of experiments 2 and 3 suggests the presence of an age factor in determining the degree of susceptibility to the agent. In experiment 2 the subcutaneous injection of 0.15 to 0.25 cc. of milk produced tumors in 69 percent of (nursing) mice 12 to 23 days old whereas 0.5 cc. caused tumors in 14 percent of animals that were 30 to 57 days old. This difference in susceptibility may be due to the difference in the dose per body weight of the older animals for 15-day-old C3H mice weigh from 7 to 9 gm. while 60-day-old animals weigh from 18 to 23 gm. In experiment 3, however, fostered C3H mice 3.5 to 4 months old were fed 0.5 to 1.0 cc. of milk and only 12 percent developed tumors, whereas in experiment 1 the feeding of 0.1 cc. of milk to mice 7 to 10 days old caused tumors in 93 percent of the animals. This difference cannot be attributed to the dose per weight relationship alone for 10-day-old C3H mice weigh from 5 to 7 gm., whereas 4-month-old animals weigh from 25 to 30 gm. It is possible that the agent may be destroyed in or not penetrate through the gastrointestinal tract of the older mice.

The dose responses reported herein cannot be compared directly with the results of others who used different strains of mice as donors and recipients of the materials administered, but their results also suggest an age factor in susceptibility to the mammary tumor inciter. Bittner (*16*) implanted pieces of spleen from strain A females into the subcutaneous tissues of F_1 hybrids derived by mating C57 Black females to strain A males. The mice were 4 to 5 weeks of age or older when inoculated, and 21.9 percent developed tumor at an average age of 15.6 months. The same technique was used for the inoculation of C3H mice 7 to 10 days old (group 9, table 1) with spleen from C3H females, and 52 percent developed tumor at an average age of 10.7 months. Bittner (*16*) also inoculated pieces of strain A lactating mammary tissue into other F_1 hybrids of the same derivation and 21.5 percent developed tumors at an average age of 15.0 months, whereas the implantation of similar tissue from strain C3H mice into C3H mice 7 to 10 days old (group 8, table 1) evoked tumors in 76 percent of the animals at an average age of 8.0 months. In another investigation Bittner (*12*) fed milk obtained from lactating strain A females to 4-week-old strain A mice. Ten consumed a total of 17 cc. of milk and 8, or 80 percent, developed tumor at an average age of 9.2 months; 10 consumed a total of 9 cc. of milk, and 3, or 30 percent, developed tumor at an average age of 10.1 months. His results from feeding a total of 9 cc. to 10 mice may be compared with the results of feeding 1.0 cc. of C3H milk to C3H females 3.5 to 4 months old (group 3, table 2), of which 8 percent developed tumor at an average age of 14.0 months. Bittner (*13*) added 30 cc. of water to 10 cc. of lyophilized spontaneous-mammary-cancer tissue from mice and fed a total of 20 cc. of the mixture to 10 strain A mice which were 4 to 5 weeks of age. Six mice developed tumor at an average age of 12.4 months. This finding is compared with the result of an experiment performed in this laboratory (*8*) in which 0.2 to 0.4 cc. of a 20-percent saline extract of fresh tumor tissue from strain C3H was fed to 46 I $\times$ C3H hybrids which were 7 to 14 days of age and 45 percent

developed tumor at a mean age of 7.8 months.[2]

As stated previously, the primary object of the work reported at this time was to ascertain whether mice develop mammary tumors if fed measured amounts of milk containing the tumor-inciting agent. The results indicate that susceptible mice 7 to 14 days old are suitable test animals for the presence of the agent when fed or injected subcutaneously. This observation together with those reported previously offer an opportunity for quantitative studies of the agent responsible for the occurrence of mammary cancer in mice. The feeding of the young mice is accomplished with ease, and the mortality is negligible. The material to be tested is fed in 0.1- or 0.2-cc. amounts, and the mice are marked individually by cutting the toes. At the time of weaning individual ear marks are given to each animal. The mice may be bred or kept as virgins. Male mice may also be utilized in the studies by the subcutaneous implantation of estrogen crystals or pellets (*18*). In our hands the most convenient method has been a single implantation of a 2-to-3-mg. pellet containing 25 percent of diethylstilbestrol in cholesterol. Mice 7 to 14 days old are uniform test animals and obviate any age factor in susceptibility suggested by the experiments reported here.

The outcome of experiment 4 reveals that hybrid mice derived by mating strain I females to strain C3H males are excellent test animals for the detection of the mammary tumor inciter in mouse milk. The high incidence of tumors in those nursing C3H foster mothers for only 24 hours shows that they are susceptible to the agent, while the absence of tumors in those obtaining milk from strain I mice only indicates that the milk from females of strain I contains little or none of the agent.

Similar to most hybrid generations obtained from inbred strains of mice, the I $\times$ C3H hybrid is vigorous and will live considerably longer than the 15-month period covered by the experiment reported here. Strain I mice develop an adenomatous lesion of the pyloric portion of the stomach (*19*), which kills many of the animals at a relatively early age, and in addition the line maintained at the National Cancer Institute has a high incidence of rectal prolapse. The first generation hybrids do not develop either lesion. Susceptibility to the stomach lesion is known to be inherited in a recessive manner (*20*). The lack of rectal prolapse in the F_1 hybrids indicates that susceptibility to this lesion is probably inherited in a similar manner.

The employment of I $\times$ C3H hybrids as test animals has done much to simplify studies on the milk agent. As stated previously, the use of fostered C3H mice involved frequent examinations for the birth of litters, the presence of lactating C57 Black mice as foster mothers, the transferring of new-born young, and in addition the use of mice from each litter as control animals. The use of hybrids obviates all these procedures. A daily examination is made for the birth of litters, and all the young can be used for experimental purposes.

Any hybrids derived by mating low-mammary-strain females to high-mammary-strain males should be satisfactory, provided the milk of the mother does not contain the agent and the hybrids are susceptible to the agent. The latter possibility must receive careful consideration, for it is known (*9*) that hybrids derived by mating strains C3H and Y are more resistant to the development of mammary

[2] *The result of this experiment as of July 15, 1942, was an incidence of 72 percent tumors at an average age of 8.9 months.*

cancer than are those obtained from strains C3H and I. Furthermore, when inbred strains of mice are crossed, it is not possible to predict the degree of susceptibility of their hybrids.

Another source of test animal is high-mammary-strain mice that have been removed from the milk agent. This should be accomplished by surgical removal of the young from the uteri of their mothers (*3*) in order to prevent any ingestion of mothers' milk. Starting with animals thus obtained, it is possible to procure a strain possessing the necessary susceptible genetic make-up minus the milk agent. The objection to this kind of test animal was noted by Bittner and by Fekete and Little. Bittner (*21*) found that after seven generations of tumor-free mice, tumors may begin to appear and concluded that "thus the line may change, in any generation, to a strain with high incidence of mammary carcinoma." It should be noted, however, that Bittner's line of mice was started from a litter of young which was born to a high-mammary-cancer-strain mother and "was given, a few hours after birth" to a low-mammary-cancer-strain foster mother. It is conceivable that a small amount of the agent was ingested by the original fostered litter. Fekete and Little (*23*) transferred fertilized ova of the high-mammary-tumor strain dba into the uteri of C57 Black mice, and the mice born from the transferred ova were nursed by the C57 Black females. Under these conditions, the dba young did not have an opportunity to ingest strain dba milk. None of the dba mice born after the ova were transferred developed a mammary tumor, but in three generations of their descendants the incidence was 11.7 percent.

A third source of test animals is the young of a strain developing few mammary cancers but possessing an inherited susceptibility to the neoplasm. Strain C mice are such animals. They develop few tumors if nursed by their own mohters, but if permitted to obtain milk from high-mammary-strain C3H foster mothers they reveal a high incidence of tumors and transmit the milk agent through successive generations (*23*).

Regardless of the derivation of the test animal, it is imperative in quantitative studies to use mice of the same genetic constitution.

SUMMARY

Milk, splenic tissues, and mammary gland from lactating strain C3H mice were administered to fostered strain C3H females.

The oral administration of 0.1 to 0.8 cc. of milk to mice 7 to 10 days old produced mammary tumors.

The subcutaneous administration of lactating mammary gland, splenic tissue, or 0.1 to 0.2 cc. of milk to mice 7 to 10 days old also produced tumors.

The subcutaneous administration of 0.15 to 0.25 cc. of milk to nursing mice produced tumors in 11 of 16 mice, whereas 0.5 cc. of milk to mice 30 to 57 days old produced tumors in 2 of 14 animals.

The oral administration of 0.5 to 1.0 cc. of milk to mice 3.5 to 4 months old produced tumors in 3 of 24 mice. Older mice are apparently more resistant to the agent than are animals 7 to 10 days old.

Mice derived from a cross of strain I females and C3H males, 7 to 14 days of age, are suitable test animals for quantitative studies on the mammary tumor inciter of mice. They are highly susceptible to the agent and do not obtain it from their strain I mothers.

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Cytology of Hepatic Tumors and Proliferating Bile Duct Epithelium in the Rat Induced With *p*-Dimethylaminoazobenzene

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INTRODUCTION

Recent investigations (*1*) demonstrated the existence of significant cytologic differences between normal hepatic cells and cells of induced and spontaneous hepatomas in the mouse. These differences, of considerable diagnostic value, consisted in a shift in the Golgi apparatus from its normal partially peripheral position to one completely juxtanuclear. The mitochondrial pattern was found to differ from that of normal hepatic tissue, showing a preponderance of one mitochondrial form in any one lesion. It was considered of importance to determine whether or not these cytologic changes correlated with neoplasia were confined to the mouse. Hence primary hepatic lesions of the rat induced with *p*-dimethylaminoazobenzene were made the subject of this investigation.

MATERIALS AND METHODS

The material was obtained from a series of 12 male Buffalo rats fed *p*-dimethylaminoazobenzene continuously for 6 months in amounts of 0.06 percent in a high fat, cystine-supplemented diet (*2, 3*) until they were killed at the age of 8 months. Special effort was made to obtain samples of hepatoma types I and II, adenocarcinoma, and bile duct proliferation from the various specimens. These lesions have been classified previously (*3*). Preparations demonstrating the Golgi apparatus were obtained by fixing the tissue immediately after the animal was killed in modified Champy fluid, 1 part 2 percent osmic acid, 1 part 3 percent $K_2Cr_2O_7$, and 1 part three-fourths percent chromic acid, for 24 hours, washing in tap water for 24 hours, and post osmicating in 2 percent osmic acid at 37° C. for from 4 to 5 days. The tissue was then inspected for completeness of impregnation by the method of Nassonov, washed, dehydrated, and embedded in paraffin in a vacuum oven. Sections of this material were cut at 4μ. Mitochondrial preparations were made from material fixed by a modified Regaud method, fixed in Regaud's fluid (4 parts 3 percent $K_2Cr_2O_7$, 1 part full strength neutral formalin), for 3 days at 3° changing each day, post chromation (after washing in distilled water) in 5 percent $K_2Cr_2O_7$ at 37° for 3 days. Paraffin sections were cut at 3μ and stained either with the Bensley-Cowdry acid fuchsin-methyl green stain or iron haematoxylin for mitochondria. All photographs of mitochondria were taken of preparations stained with iron haematoxylin.

RESULTS

The Golgi Apparatus

Considerable difficulty was experienced in obtaining satisfactory preparations of the Golgi apparatus in this material owing to the presence of large amounts of lipoid. This is a common feature of the livers of rats maintained on the foregoing dietary regimen (*3*). However, adequate preparations were obtained in sufficient quantity so that the following descriptions of the Golgi apparatus of hepatoma types I and II, adenocarcinoma, and proliferating bile duct epithelium may be considered typical for each lesion.

Hepatoma Type I

The normal distribution of the Golgi material in the hepatic cells of the rat is similar to that in the mouse. The greater portion is in a peripheral position concentrated near the adjacent bile capillary, while small additional juxtanuclear strands are also present (fig. 1). As in hepatic tumors in the mouse (*1*), the Golgi apparatus present in cells of hepatoma type I was found to be completely juxtanuclear in position. The amount of Golgi material appeared to be somewhat reduced (fig. 2). Some variation in amount and extent of the Golgi material from lesion to lesion occurred, but figure 2 is representative.

Hepatoma Type II

In hepatoma type II the Golgi apparatus was also found to be juxtanuclear in position and somewhat similar to that in hepatoma type I (fig. 3). The type of Golgi apparatus in this lesion is very similar to that in the cells of rat hepatoma 31 described previously (*4*).

Adenocarcinoma

In typical adenocarcinoma, the epithelium varies from low to tall columnar cells. The low columnar cells possess a completely juxtanuclear Golgi apparatus similar to that found in hepatoma type II. In tall columnar cells, the Golgi apparatus appears to be pulled out along the long axis of the cell, being entirely distal to the nucleus (fig. 4). In certain areas the pattren is reminiscent of that found in the cells of normal intestinal epithelium.

Proliferating Bile Duct Epithelium

In the cells of proliferating bile duct epithelium, the Golgi material was rather small in amount when compared with that of adenocarcinoma cells and tended to extend down around the lateral borders of the nucleus as smooth strands (fig. 5). It was found that cells of proliferating bile duct epithelium were readily distinguishable from adenocarcinoma cells by the differences in the Golgi apparatus, and transitional forms between the two cell types were not found.

Mitochondria

The predominant type of mitochondria present in the normal rat hepatic cell is dependent at least in part upon the position of the cell within the hepatic lobule (*5*). Preponderantly spherical forms are present near the periphery, and filamentous forms near the central vein of the lobule (fig. 6, *A* and *B*). In any one neoplasm of the rat liver the mitochondria tend to be of one size and type. Figure 7 shows the spherical mitochondria present in cells of hepatoma type I which is the neoplasm most nearly resembling normal hepatic tissue (*3*). Rarely, small nodules of this hepatoma occur which contain extremely large, plump, rodlike mitochondria (fig. 8). Little variation was noted in the mitochrondria in hepatoma type II. The typical form was a very

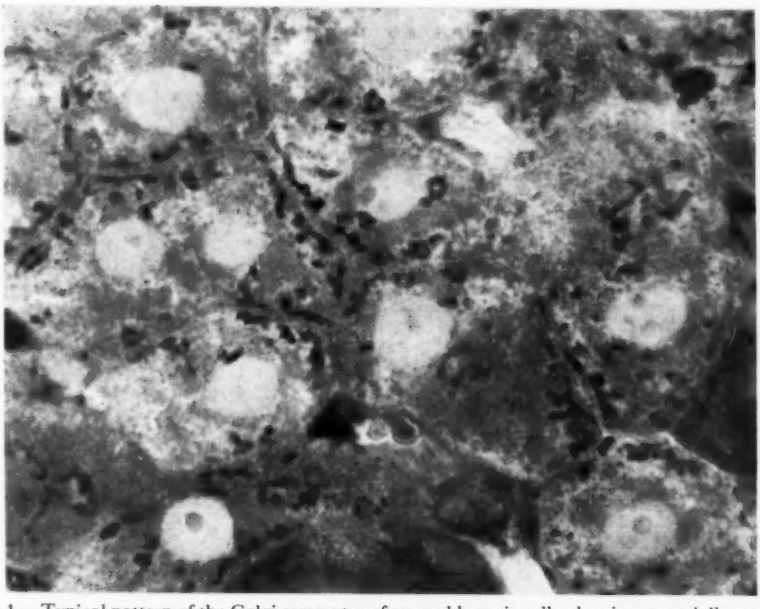

FIGURE 1.—Typical pattern of the Golgi apparatus of normal hepatic cells, showing a partially peripheral network outlining the bile capillaries. Smaller juxtanuclear portions are also evident. × 1300.

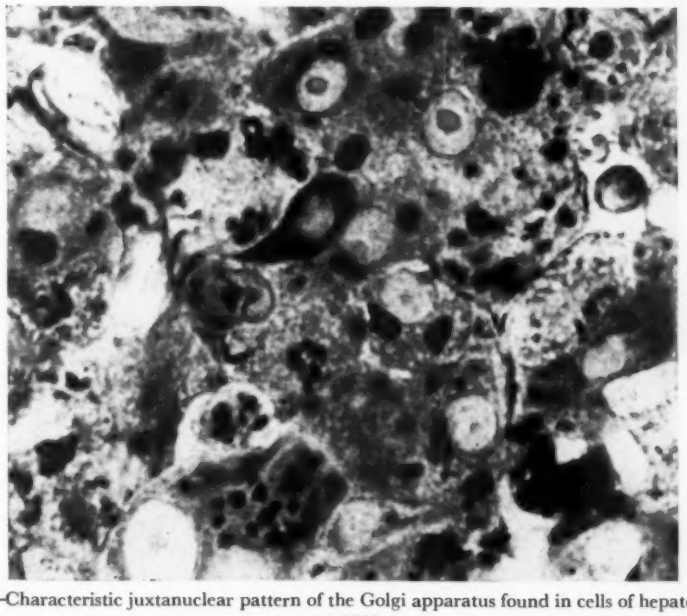

FIGURE 2.—Characteristic juxtanuclear pattern of the Golgi apparatus found in cells of hepatoma type I. × 1300.

fine, tenuous, filamentous type, sometimes beaded, sometimes smooth. These mitochondria are so slender that they are very difficult to differentiate and photograph (fig. 9).

Adenocarcinoma cells were, as a rule, readily distinguishable from cells of proliferating bile duct epithelium in mitochondrial preparations. In most sections of this tumor some highly differentiated cells were present, possessing terminal bars and a striated border (fig. 10). In addition the characteristic tenuous filamentous mitochondria were concentrated near the distal border of the cell between the nucleus and the terminal-bar apparatus. Just beneath the terminal-bar apparatus a zone free of mitochondria was frequently visible. The characteristic picture seen in bile duct proliferation showed no such differentiation (fig. 11), the distal cell boundary being perfectly smooth. Occasionally (fig. 12), a terminal-bar apparatus was visible, but a striated border was not seen, nor was there any evidence of a distal concentration of mitochondria.

DISCUSSION

These results indicate that in the rat as in the mouse, the shift in the Golgi apparatus to a completely juxtanuclear position characterizes the cells of hepatic neoplasms. As indicated previously (*1*), this shift in the position of the Golgi apparatus in mouse hepatomas may well be correlated with lack of evidence as to the presence of bile pigment. A similar relationship appears to exist in the *p*-diemthylaminoazobenzene-induced hepatic tumors in the rat since Edwards and White (*3*) found no evidence of bile secretion in these lesions, contrary to the report of Kinosita (*6*) who stated that bile pigments were

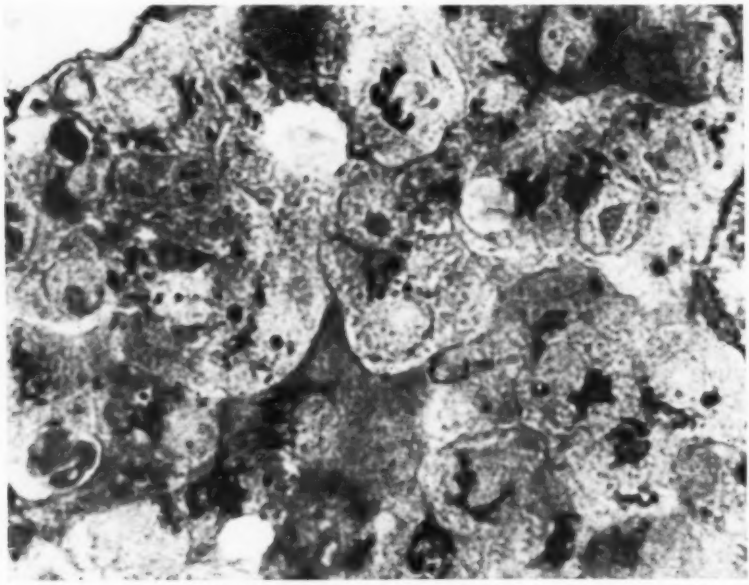

FIGURE 3.—Golgi apparatus found in cells of hepatoma type II—juxtanuclear and somewhat greater in amount than that in cells of hepatoma type I. ×1300.

FIGURE 4.—Golgi apparatus of adenocarcinoma. In low columnar cells the Golgi apparatus is juxtanuclear and similar to that of hepatoma type II. In tall columnar cells it appears similar to that of normal intestinal epithelium. ×1300.

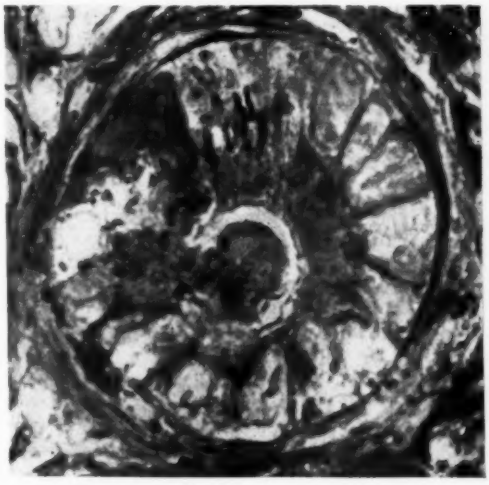

FIGURE 5.—Proliferating bile duct epithelium. The Golgi apparatus appears to consist partly of smooth strands which frequently extend down around the lateral borders of the nucleus. Compare with figure 4. ×1300.

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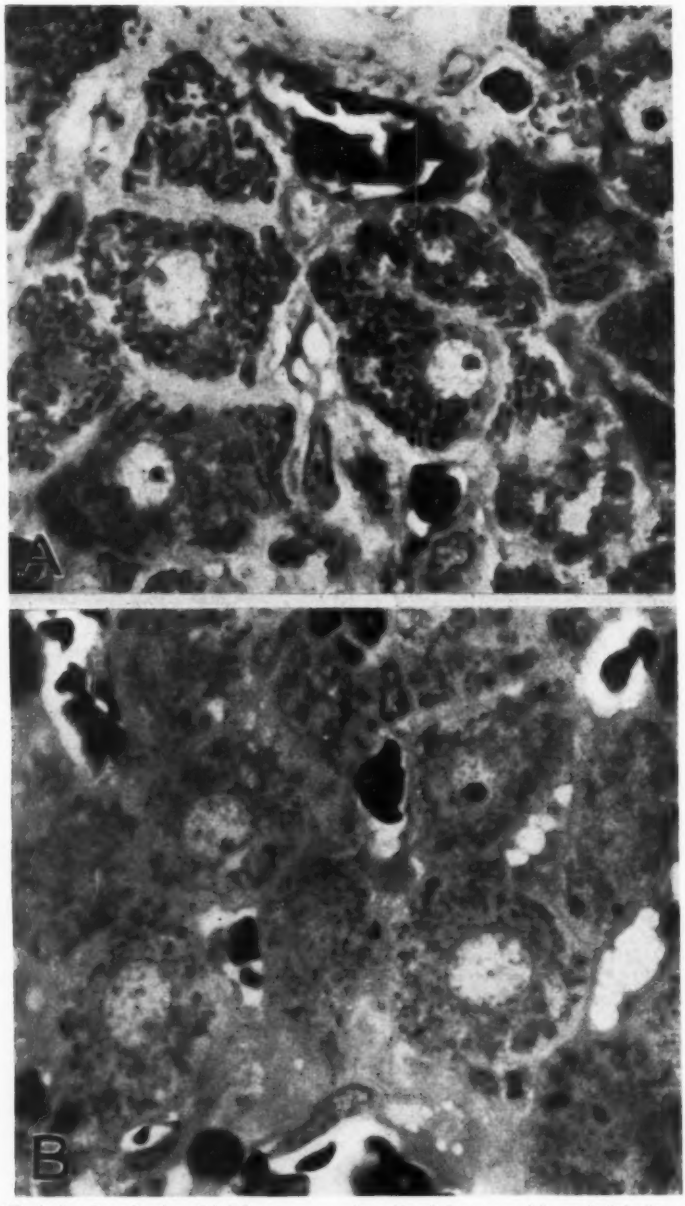

FIGURE 6.—Variation in mitochondrial form present in cells of the normal hepatic lobule. *A*, Spherical forms in cells near the periphery of the lobule near the portal canal. *B*, Filamentous forms in cells near the central vein of the lobule. *A* and *B*, ×1300.

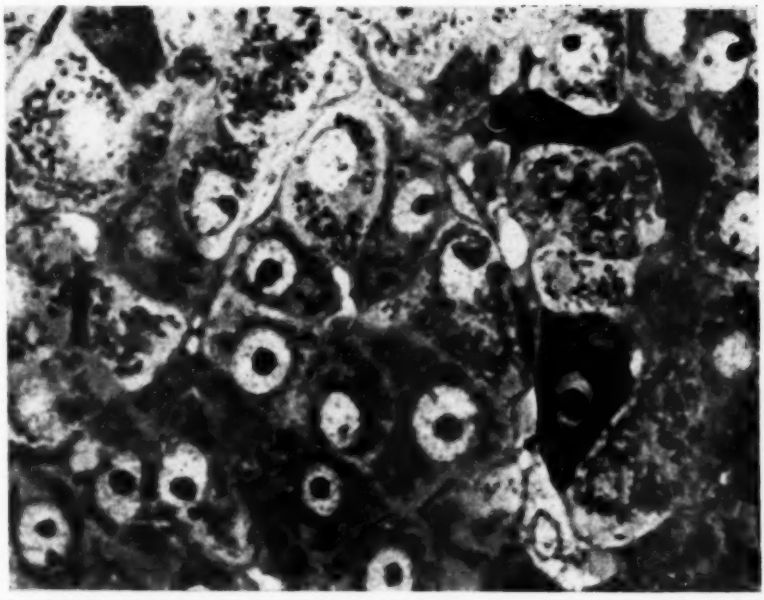

FIGURE 7.—Spherical mitochondria present in the cells of the majority of hepatomas of type I. ×1300.

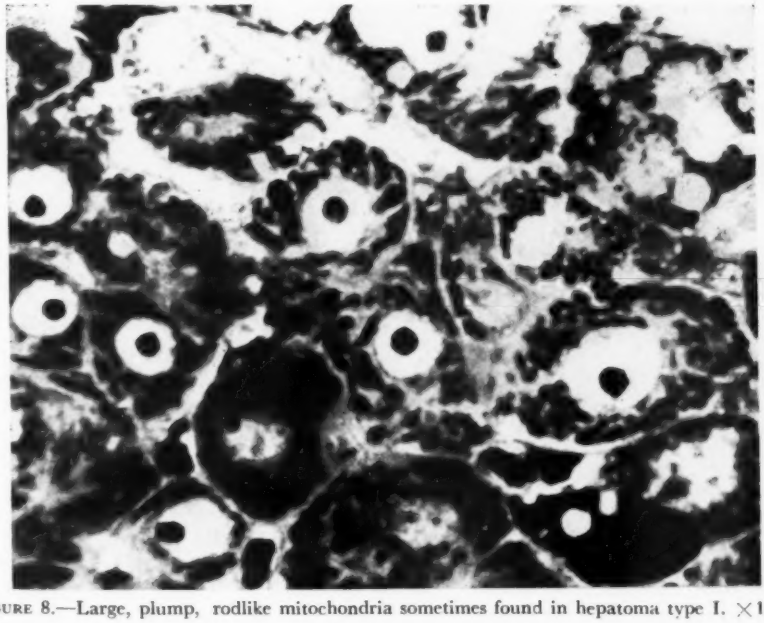

FIGURE 8.—Large, plump, rodlike mitochondria sometimes found in hepatoma type I. ×1300.

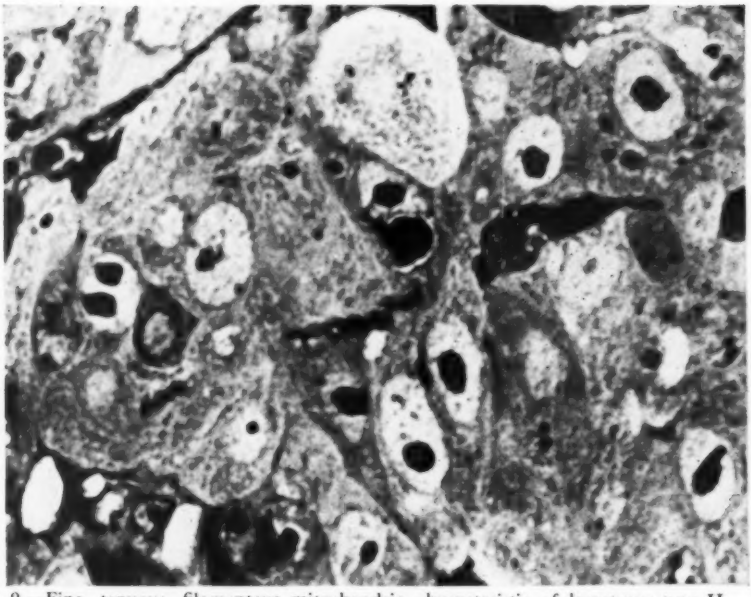

FIGURE 9.—Fine, tenuous, filamentous mitochondria characteristic of hepatoma type II. × 1300.

FIGURE 10.—Highly differentiated adenocarcinoma showing a distal concentration of filamentous mitochondria. A zone free of mitochondria is just proximal to a clearly defined terminal-bar apparatus. A striated cuticular border is also present. × 1300.

FIGURE 11.—Cells of proliferating bile duct epithelium showing relatively few scattered mitochondria and no striated border. ×1300.

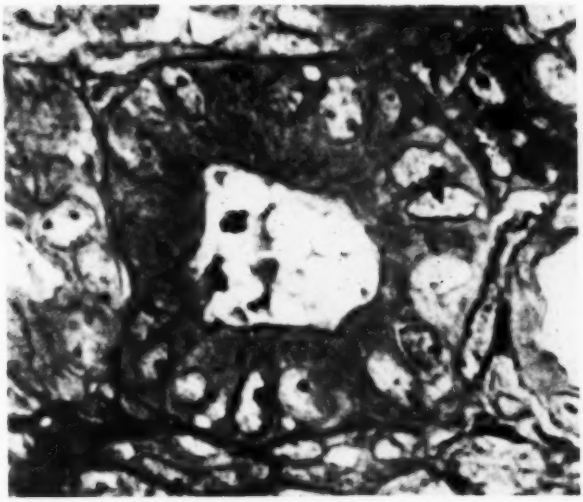

FIGURE 12.—Well-differentiated bile duct epithelium showing fairly numerous, evenly distributed mitochondria and a terminal bar apparatus, but no striated border. ×1300.

present in induced hepatomas of the rat. The frequent occurrence of green pigment in primary cancer of the human liver suggests that bile pigment is commonly present in such neoplasms. It will be of considerable interest to study the Golgi apparatus of human hepatoma cells and thus obtain evidence as to whether the shift in the Golgi apparatus is related to neoplasia as such or to the change in function which appears to occur in mouse and rat hepatomas.

The presence of a preponderant mitochondrial type in a given tumor holds for the rat as well as the mouse. Ususally spherical mitochondria are found in hepatoma type I and filamentous forms in hepatoma type II. Exceptions to these findings have been noted, but they are rare. Nodules of hepatoma type I with extremely large, plump, rodlike mitochondria occur. There is no reason to believe that such forms are the result of faulty fixation since the size of all such mitochondria are well beyond the limits found for mitochondria in well or poorly fixed normal hepatic tissue. The significance of this finding is not apparent, but it is of interest that the great majority of the mitochondria in such lesions are well above the normal size.

The cells of hepatoma type II and of rat hepatoma 31 being carried in this Institute are nearly identical cytologically, yet the primary tumor from which hepatoma 31 was obtained was mixed hepatoma types I and II and adenocarcinoma. It is suggested that the tissue which grew out on transplantation was hepatoma type II.

The cytologic study of hepatic adenocarcinoma in the rat has added some evidence which may help to determine the cell of origin of this tumor. Maruya (*7*) and Kinosita (*6*) believe it to be derived from hepatic parenchymal cells, while Orr (*8*) considers it a tumor of bile duct epithelium. While presenting evidence favoring the former view, Edwards and White (*3*) state that "the cell of origin of the induced adenocarcinoma of the liver has by no means been directly established." Orr (*8*) based his opinion partly on cytologic evidence, while Kinosita based his on evidence of transitions between the hepatic parenchymal cell and frank adenocarcinoma. On the other hand, Edwards and White found no evidence of transition between bile duct epithelium and adenocarcinoma. A comparative study of the finer cytologic structure of adenocarcinoma, proliferating bile duct epithelium, and the hepatic parenchymal cell does not support either of these views directly. However, of the two possible cells of origin, the Golgi apparatus of the adenocarcinoma cells more nearly resembles that of the hepatic parenchymal cell. In fact, it is much like the apparatus of normal intestinal epithelium. In the present study intermediate types of Golgi apparatus between that of hepatoma type II and adenocarcinoma were occasionally noted, and in addition many of the low columnar cells in adenocarcinoma possessed a Golgi apparatus similar to that in cells of hepatoma type II. Transitional cell types between hepatoma type II and adenocarcinoma were found previously (*3*), yet no such transitional forms between adenocarcinoma cells and cells of proliferating bile duct epithelium have been observed.

In mitochondrial preparations, the similarity between the most highly differentiated cells of the adenocarcinoma and the cells of normal intestinal epithelium was again observed. A striated, cuticular border was present, with an associated well-developed terminal-bar apparatus. Immediately beneath this was a zone free of mitochondria. The mitochondria were filamentous distal to the nucleus. All these

cytologic characteristics are also those of normal intestinal epithelium. No such specialization was evident in cells of proliferating bile duct epithelium. While it is for the most part indirect, the cytologic evidence favors the view that adenocarcinoma originates from hepatic parenchymal cells by way of hepatoma type II rather than from bile duct epithelium.

SUMMARY

A cytologic study of various lesions of the livers of 12 Buffalo rats fed *p*-dimethylaminoazobenzene has been made.

In all neoplasms derived from the hepatic parenchymal cell, the Golgi apparatus shifts to a completely juxtanuclear position.

The type of mitochondria varies from lesion to lesion, one mitochondrial form predominating in any one neoplasm.

Spherical mitochondria are characteristic of hepatoma type I, although rare and sometimes bizarre exceptions occur.

Tenuous, fine, filamentous mitochondria are characteristic of hepatoma type II.

Cytologic evidence indicates that the transplantable hepatoma 31 originated as a primary hepatoma of type II.

From the evidence presented, it is suggested that the hepatic adenocarcinoma of the rat is derived from hepatic parenchymal cells rather than from cells of proliferating bile duct epithelium.

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Intestinal Adenocarcinoma and Intra-Abdominal Hemangio-Endothelioma in Mice Ingesting Methylcholanthrene

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Lorenz and Stewart reported the production in mice of squamous-cell carcinoma of the forestomach, as well as intestinal carcinoma, by the oral administration of 1,2,5,6-dibenzanthracene and 20-methylcholanthrene (*1–3*). The carcinogenic agent was administered in an aqueous olive-oil or a mineral-oil emulsion, and these preparations were given to the animal ad libitum in place of drinking water.

The purpose of this paper is to report the induction of similar lesions and of hemangio-endothelioma in mice by incorporating 20-methylcholanthrene in the food rather than in the drinking water.

MATERIALS AND METHOD

The animals used were 2-month-old C3H mice of the Andervont line equally divided as to sex, and strain C mice of the same age and sex distribution which were obtained from the local colony of strain C mice bred by Dr. H. B. Andervont. From the time of weaning to the beginning of the experiment they had been maintained on Purina dog chow pellets. When the experiment was begun they were put on the following dietary regimen:

	Percent
Casein	6
Corn starch	45
Crisco	23.5
Sucrose	15
Cod-liver oil	1
Inorganic salts [1]	4
Brewers' yeast [2]	5
l-Cystine	.5
	100.0

[1] Modified Osborne-Mendel salt mixture.

[2] Harris' dried brewers' yeast.

To every 100 gm. of this dietary mixture 55 mg. of 20-methylcholanthrene was added by dissolving the hydrocarbon in melted Crisco. This solution was then mixed with the dry ingredients. Each mouse consumed 2 to 3 gm. of food per day depending on its size. Many of the animals died within a period of 3 to 4 months on this regimen because of the lack of tolerance for this diet. The mice which survived on the experimental diet for 6 months were placed on the basal diet to which no methylcholanthrene had been added. By this procedure it was possible to prolong the life of the animals and thus permit any small tumors to gain an easily recognizable size.

PATHOLOGIC OBSERVATIONS

INTESTINAL CARCINOMA

As expected, there was a high incidence of adenocarcinoma and precancerous lesions of the small intestine in the mice ingesting methylcholanthrene. The definition of the term "precancerous lesion" as it is used here is identical with that reported in another communication (*4*) from this Institute. Of 50 C3H mice ingesting the methylcholanthrene, autopsy revealed 6 with adenocarcinoma of

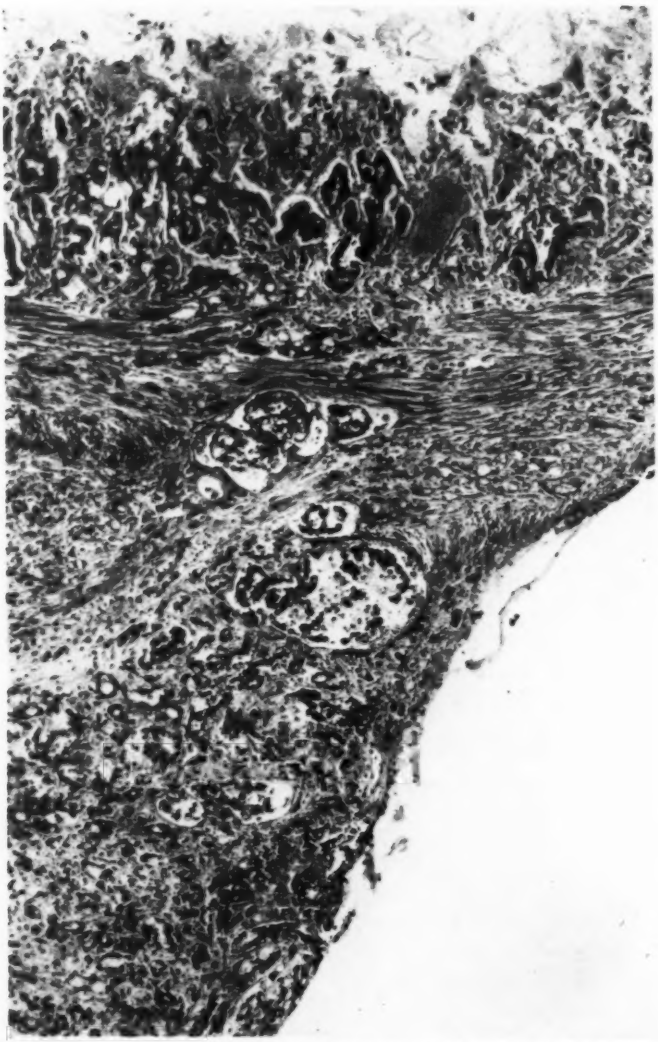

FIGURE 1.—Small intestinal adenocarcinoma with involvement of muscularis and extensive infiltration of mesentery. × 80.

the small intestine alone; 6 had combined adenocarcinoma and precancerous lesions of the small intestine; and 18 had only precancerous lesions of the small intestine. Of the mice with adenocarcinoma of the small intestine, 4 had a single tumor; and in 2 mice the tumors were multiple.

Of the 32 strain C mice ingesting the methylcholanthrene, 4 had adenocarcinoma of the small intestine alone, and 5 had only precancerous lesions of the small intestine. None had combined adeno-

carcinoma and precancerous lesions of the small intestine. Of the 4 C mice with adenocarcinoma of the small intestine, 3 had a single tumor and 1 had 2 tumors.

In the strain C mice the earliest precancerous lesions of the small intestine were observed 196 days after the experiment was begun and the earliest adenocarcinoma after 203 days, while in the C3H mice the first precancerous lesion was seen after 60 days and the first intestinal carcinoma after 109 days. The precancerous lesions averaged 2–4 mm. in diameter. The carcinomatous lesions ranged from 2 to 11 mm. and averaged 5 mm. in diameter. The distribution and the gross and microscopic characteristics of the precancerous and carcinomatous lesions of the small intestine were similar in the two strains of mice (fig. 1).[1] Most of them occurred at an average distance of 10–18 cm. from the pyloris with a spread from 8–25 cm. Metastases occurred in five mice as follows: Mesenteric lymph node, 4; mesentery, 4; liver, 1; and lung, 1. The metastases in the mesentery and mesenteric lymph node were similar to those described in the previous publication (*1*), with the exception that in one mouse with metastasis to the mesentery there was secondary infiltration of the serosa and muscularis of the wall of the stomach. In this mouse, the neoplastic infiltration of the stomach was proceeding from without inward, and the mucous membrane of the stomach was completely intact and appeared normal. The intestinal tumor of one mouse which showed metastasis was considerably more anaplastic than the majority of the tumors so far studied. In the mouse in which the intestinal carcinoma metastasized to the mesenteric lymph node, mesentery, liver, and lung, a study of the primary tumor revealed extensive carcinomatous permeation of the lymphatic vessels in the deep portion of the submucosa (figs. 2, 3, and 4). One section also showed a markedly dilated blood vessel containing a tumor embolus. In this case there was also a hemangio-endothelioma of the intestine in close proximity to the intestinal adenocarcinoma, and the possibility exists that the large blood channels in the

[1] *Unless otherwise stated all the photomicrographs shown were prepared from sections stained with haematoxylin and eosin.*

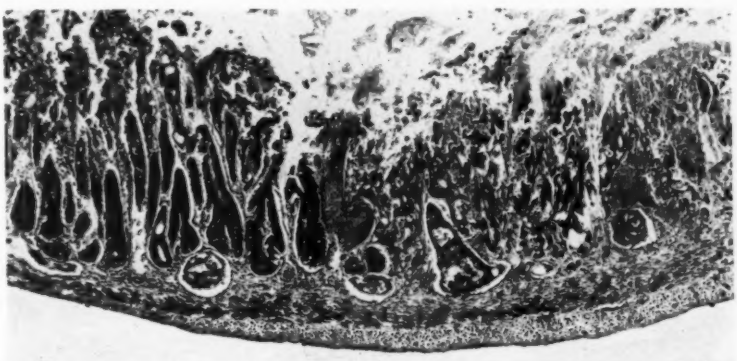

FIGURE 2.—Small intestinal adenocarcinoma. Tumor has as yet not invaded the muscularis but there is extensive invasion of submucosal lymphatics. ×63. See metastases from this tumor in figures

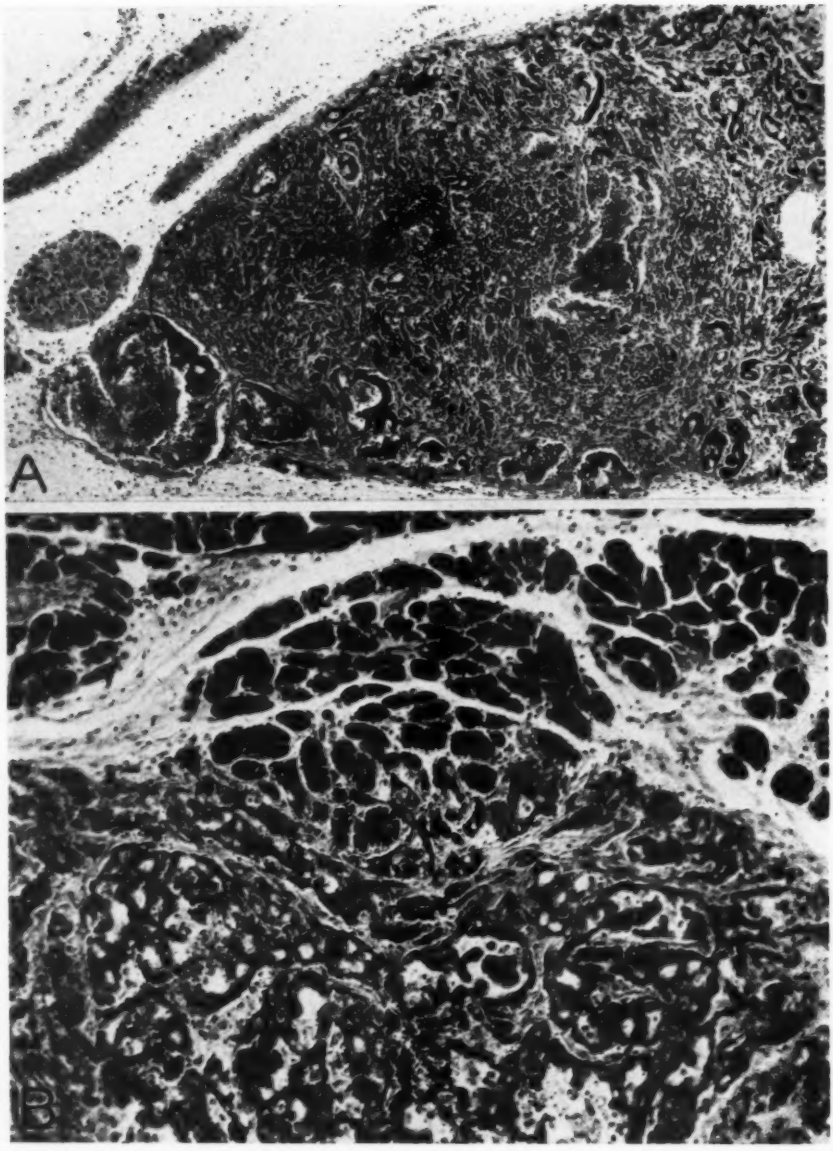

FIGURE 3.—Metastases from carcinoma illustrated in figure 2. *A*, Mesenteric lymph node. ×63; *B*, Mesentery with invasion of pancreas ×117.

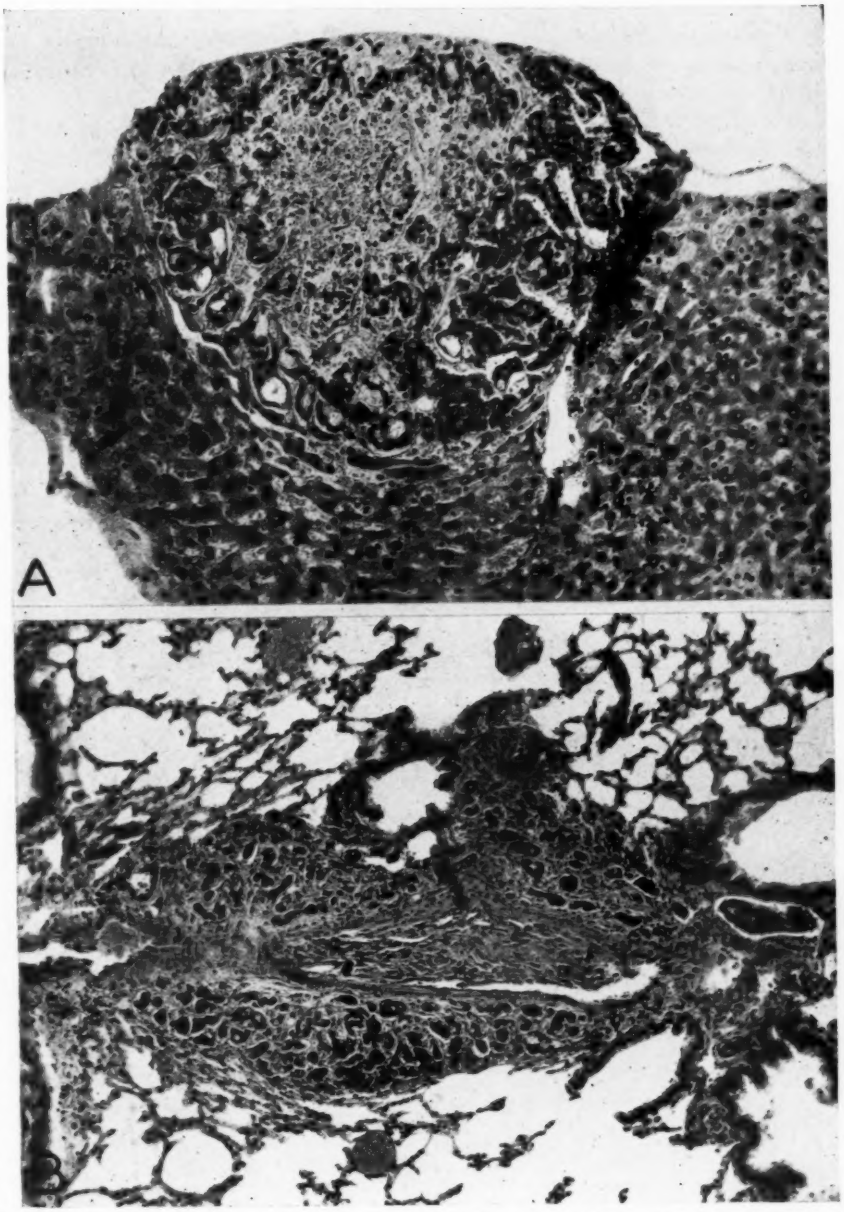

FIGURE 4.—Metastases from carcinoma illustrated in figure 2. *A*, Liver. ×155; *B*, Lung. ×110.

vascular tumor may have afforded an easily accessible route for metastasis. The pulmonary metastasis in this case could be readily distinguished from the common spontaneous and induced pulmonary tumor of the mouse, since the former showed permeation of the peribronchial and perivascular lymphatics in contrast with primary pulmonary tumors which usually do not show this feature. The hepatic metastases were multiple and occurred both near the surface and in the depth of the organ. Under the conditions of this experiment intestinal adenocarcinomas were more frequent in the C3H than in the strain C mice. This is in contrast with the hemangio-endotheliomas, for which the reverse was true.

Intestinal and Mesenteric Hemangio-Endothelioma

Hemangio-endotheliomas occurred in 11 strain C3H mice and in 21 strain C mice, the lesion being equally frequent in males and females. All except 1 tumor occurred in 1 or more of the following sites: small intestine, mesentery, pancreas, or mesenteric lymph node; there was metastasis to the liver in 1 case, to the lung and retroperitoneal lymph node in another, and several tumors were adherent to the large intestine. One tumor was apparently primary in the penis.

The first hemangio-endothelioma observed in strain C was 174 days after the beginning of the experiment and the first in strain C3H was at 168 days. The intestinal hemangio-endotheliomas varied in size from 1 to 16 mm. in diameter, with an average of 5 mm. When the lesion was situated in the mesentery or one of its associated structures, the tumor varied in diameter from 2 to 22 mm. and averaged 9 mm.

The hemangio-endotheliomas confined to the small intestine appeared as one to several small, round, petechialike, semianular, anular, constricting lesions, or as pedunculated masses (fig. 5, *A*). They were predominantly primarily serous in location and occurred along the mesenteric attachment or in the contra-mesenteric portion of the small intestine. Many of the tumors did not penetrate deeply from the serosa into the wall of the intestine (figs. 5, *B*, and 6). Others, however, penetrated all coats, with resulting ulceration of the mucous surface or the formation of a neoplastic nodule extending into the lumen of the intestine, causing obstruction in some cases. In a few instances, a hemangio-endothelioma and an adenocarcinoma of the small intestine occurred in close proximity to each other or at opposing sites, the one on the serosal, the other on the mucosal surface. Under these circumstances, the hemangio-endothelioma could usually be distinguished by its red color in contrast with the gray appearance of the carcinoma. In a few instances, these lesions were so closely associated that each entity could be disassociated only after careful microscopic study.

Many of the hemangio-endotheliomas occurred as mesenteric masses primary in the mesentery (fig. 7), pancreas, or mesenteric lymph nodes. When large, these growths also extended to involve the neighboring large or the small intestine. In those cases in which the colon was infiltrated by a mass in common with the mesentery, it was considered doubtful that the lesion had arisen in the large intestine, since in no instance in this series was a primary hemangio-endothelioma restricted to the colon. However, when the small intestine was involved, it was impossible to state the origin of the tumor. The mesenteric tumors were large, firm, irregular, nodular, red, brown, or dark in color.

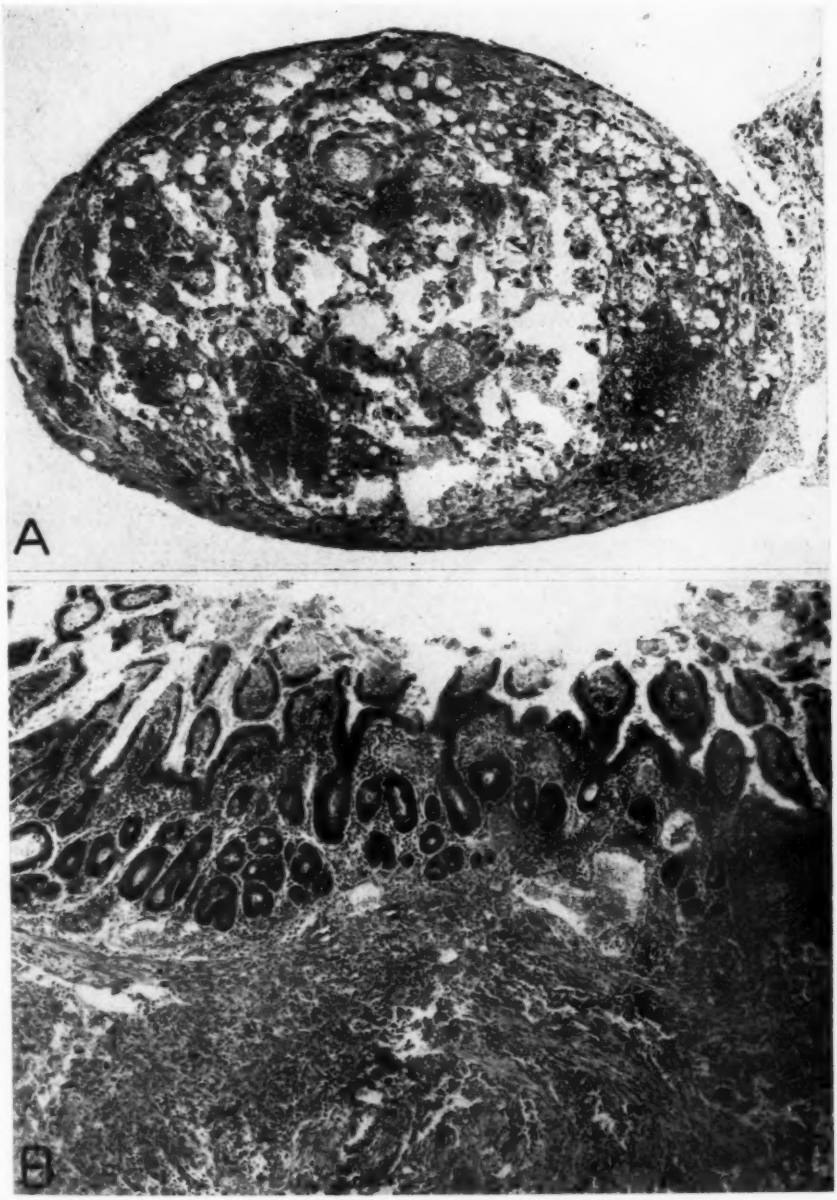

FIGURE 5.—*A*, Pedunculated hemangio-endothelioma which arose in the intestinal serosa. × 63; *B*, Hemangio-endothelioma of small intestine involving muscularis and submucosa. Mucosa intact. × 63.

Some of them were roughly shaped like a hand with stublike fingers which extended out toward the intestinal border of the mesentery, sometimes reaching and invading the intestine. They were located in various areas of the mesentery, along the colon, among the coils of the small intestine, and embraced by the curve of the duodenum. In some specimens with hemangio-endothelioma of the mesentery, portions of the pancreas and mesenteric lymph node were definitely free of tumor while in other specimens these were involved. One mouse with hemangio-endothelioma of the mesentery and intestine had a ruptured small intestine.

Microscopically, the hemangio-endotheliomas irrespective of location were similar structurally within the limits of variation characteristic of any given tumor. The cellularity varied considerably, as did also the size, shape, number, and appearance of the vascular channels. Some parts of the tumor were composed almost completely of closely packed, thin-walled, blood-filled, vascular channels varying in width from capillary to cavernous spaces (fig. 8) interspersed with sheets of atypical endothelial cells. Other areas were chiefly cellular, with and without cleftlike blood spaces. The blood spaces of the tumor were lined by endotheliallike cells which were often stratified (fig. 9, *A*) and frequently observed in mitosis. With the phosphotungstic acid-haematoxylin stain, a few short blue fibrils were occasionally observed in some of the tumor cells, but this feature was not constant. In the vascular areas the nuclei of the tumor cells were hyperchromatic, frequently single, rounded, oval, or elongated, but some were markedly irregular in size and shape, being folded, wrinkled, lobated, and exceedingly large and multiple and contained large masses of chromatin. The giant cells sometimes appeared like multi-

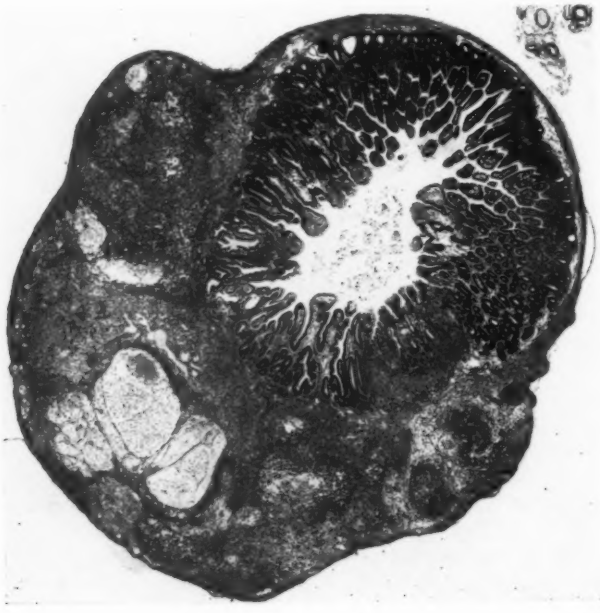

FIGURE 6.—Hemangio-endothelioma of small intestine showing extensive involvement of deeper layers of gut. Except for two foci mucosa has been left intact. × 20.

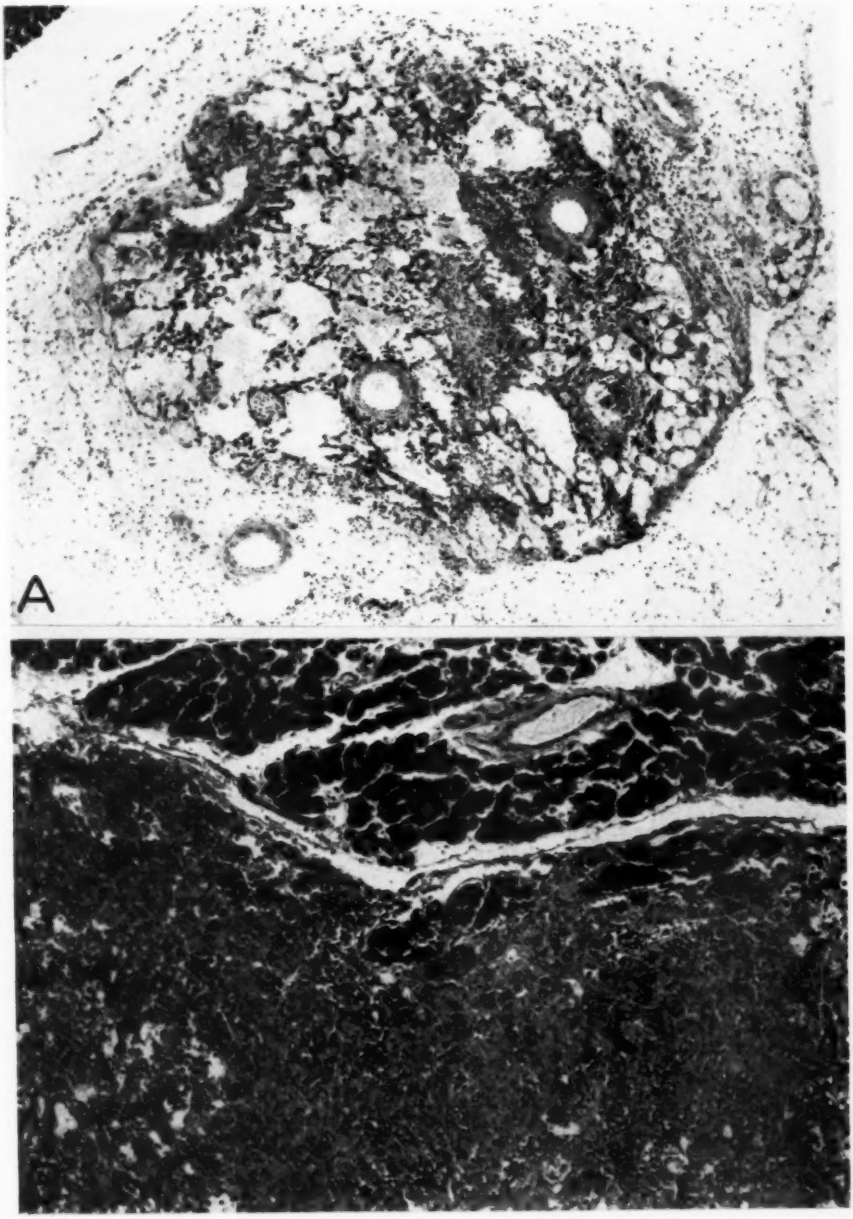

FIGURE 7.—*A*, Small hemangio-endothelioma of mesentery. Lesion is largely vascular. × 30; *B*, Hemangio-endothelioma of mesentery with invasion of pancreas. Lesion has both solid and vascular areas. × 28.

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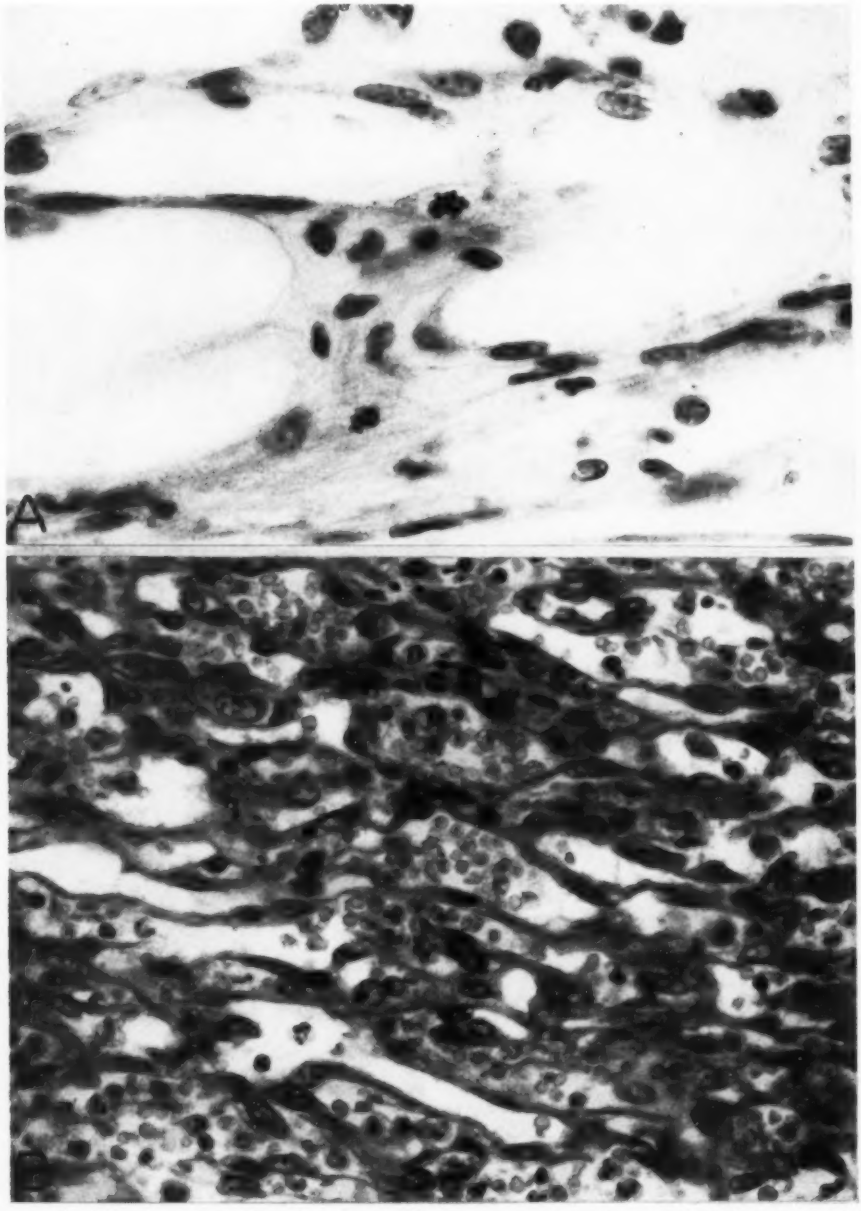

FIGURE 8.—Vascular hemangio-endothelioma. Note numerous prominent endothelial nuclei often bulging into lumen of channel. *A*, × 866; *B*, × 285.

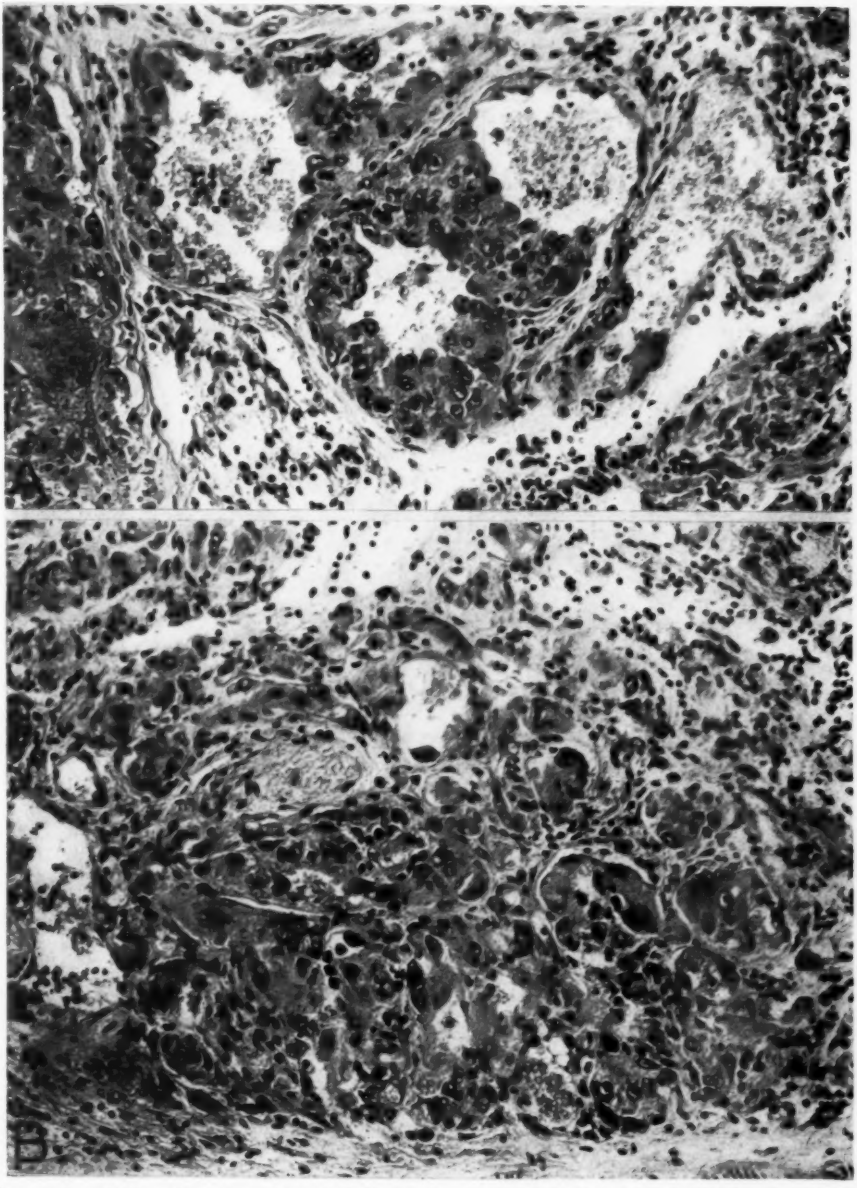

FIGURE 9.—*A*, Vascular hemangio-endothelioma showing stratification of endothelial cells. × 235; *B*, Vascular hemangio-endothelioma with intraluminal proliferation of endothelial cells. × 235.

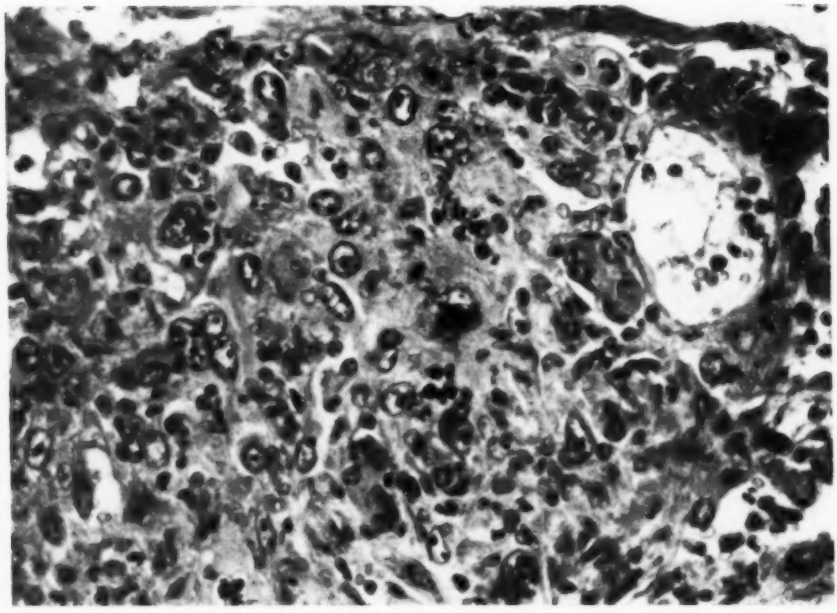

FIGURE 10.—Solid area in hemangio-endothelioma. × 260.

nucleated foreign-body giant cells and at other times possessed a ramifying, lobated nucleus resembling a megakaryocyte. There were usually one to two mitotic figures per high-power field, many of which were atypical. Various degrees of intravascular endothelial cell proliferation were present—from a single or double layer of endothelial cells up to the point where a vessel was partially or completely occluded by cell proliferation (fig. 9, *B*). The lumina of most of the vessels contained blood, but some were empty. Some vessels were thrombosed, and some contained large numbers of polymorphonuclear leukocytes. There were numerous inflammatory foci and necrotic areas throughout the tumor.

Some of the tumor cells in the solid areas were fairly uniform, with oval, pale, vesicular nuclei, and some cells were spindle-shaped (fig. 10). The giant cells were numerous, with an abundance of deeply acidophilic cytoplasm, sometimes vacuolated and foamy, with phagocytosed material such as pigment and fragments of nuclei. The majority of the cells, including practically all the spindle-shaped cells, were devoid of fibrils. In some areas the cells were arranged in sheets. The line of demarcation between the tumor and surrounding tissues was often rather sharp, but there was no limiting capsule, and active invasion of the surrounding tissue occurred. The reticulum distribution in these tumors was characteristic of hemangio-endothelioma. In the vascular areas the reticulum fibers occupied a basement-membranelike position with respect to the vessel (fig. 11, *A*), while in those areas containing solid areas this substance surrounded cellular aggregation (fig. 11, *B*). The association of reticulum with individual cells in such areas was entirely haphazard.

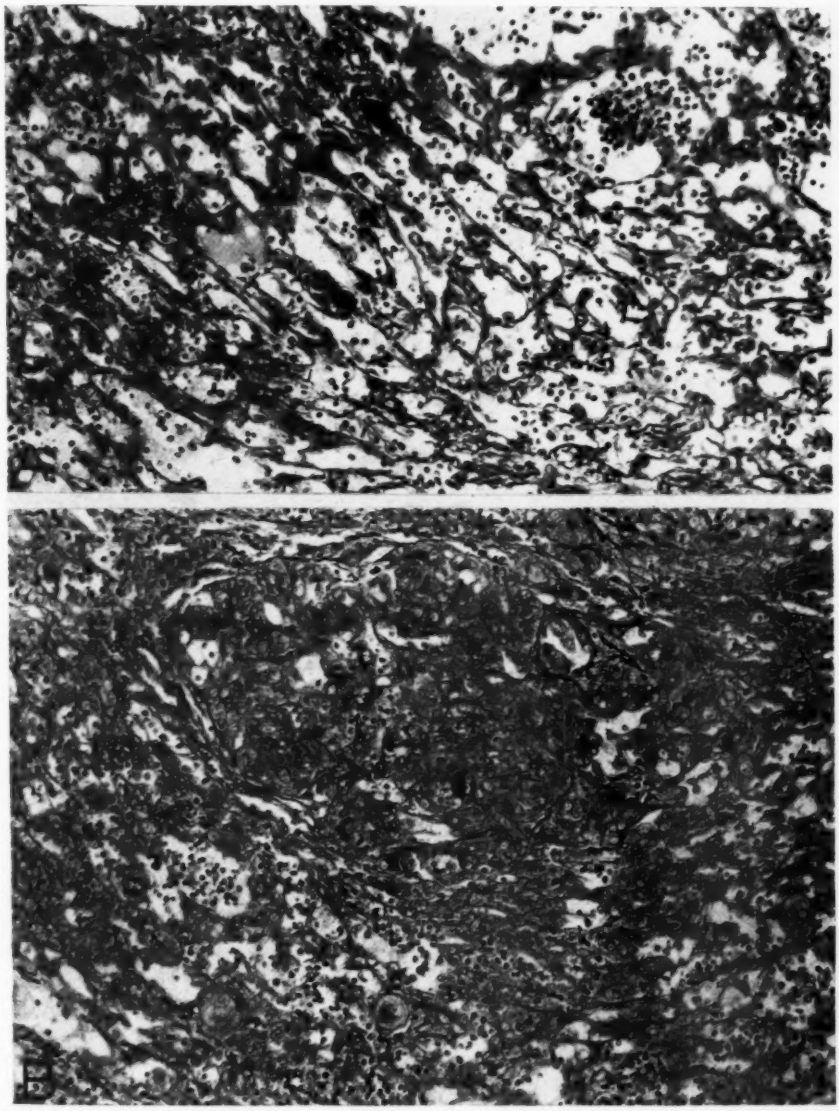

FIGURE 11.—*A*, Vascular hemangio-endothelioma. Reticulum occupies a basement-membranelike position with respect to the vessels. Laidlaw's stain for reticulum. × 195; *B*, The reticulum is distributed at the margin of a solid area in a hemangio-endothelioma. Laidlaw's stain for reticulum. × 180.

When the hemangio-endothelioma was situated within the mesentery, it also frequently involved the pancreas and the mesenteric lymph node even when the tumor was spatially small. In other cases the lesion was confined to one or another of these structures, and in still other cases two or more were involved by the tumor. The metastatic lesions in the liver and lung resembled the primary tumors.

In those cases in which an adenocarcinoma and a hemangio-endothelioma were in close proximity or involved opposing sites of the intestine, the former infiltrating from the mucous and the latter from the serous membrane, the two histologic types remained separate and distinct even though they formed a collision tumor.

Gastric Lesions

Papillomas, hyperkeratosis, hypertrophy, or hyperplasia of the mucous membrane of the forestomach occurred in 13 strain C mice and 18 strain C3H mice. In addition, 1 strain C3H mouse developed a squamous-cell carcinoma of the forestomach. The first forestomach papilloma observed in a strain C mouse was at 156 days after the start of the experiment, and the first in a strain C3H mouse at 144 days. The gross and microscopic appearance of these lesions were similar to those previously described in publications from this Institute (*2*, *3*). In the majority of the cases the papillomas varied from 1 to 5 mm. in diameter and were frequently multiple. Since we set up as our criterion for the diagnosis of primary cancer of the stomach that the tumor shall have penetrated all coats of the viscus and shall have formed a neoplastic nodule on the serosa, we have classified as papillomas many tumors which actually showed permeation of the submucosa with limited infiltration of the upper muscularis. It is probable that some of these latter lesions were either potentially malignant or were actually early carcinomas.

Peritoneal Effusion

Ascites occurred frequently in both the C3H and C strains of mice. In many cases this occurrence was also associated with bilateral pleural effusion and extensive subcutaneous edema. In a number of the mice the ascitic fluid was chylous in nature. Hemorrhagic peritoneal effusions occurred only in strain C mice; and since the occurrence was usually associated with an abdominal hemangio-endothelioma, it is considered that this tumor was the source of the blood in the case of the hemorrhagic peritoneal effusions. There appears to be no obvious reason for the absence of the hemorrhagic effusions in the strain C3H mice with peritoneal hemangio-endotheliomas.

Hepatic Lesions

The livers of most of the experimental mice of both strains showed a marked fatty change during the period while they were ingesting methylcholanthrene and the basal diet. When the animals were placed on the basal diet alone, the fatty change disappeared. Fatty change of the liver has not been a regular feature in other methylcholanthrene-feeding experiments in which the hydrocarbon was administered as aqueous oil emulsions. This may be attributed in part to the basal diet employed inasmuch as the diet was low in protein and high in fat. In the fatty livers of some of the mice, stainable lipoidal material was found in the paraffin sections. Two mice had slight cirrhosis, and one of them had multiple hepatomas.

Miscellaneous Lesions

Eleven strain C3H mice and one strain C mouse developed one or more calcific

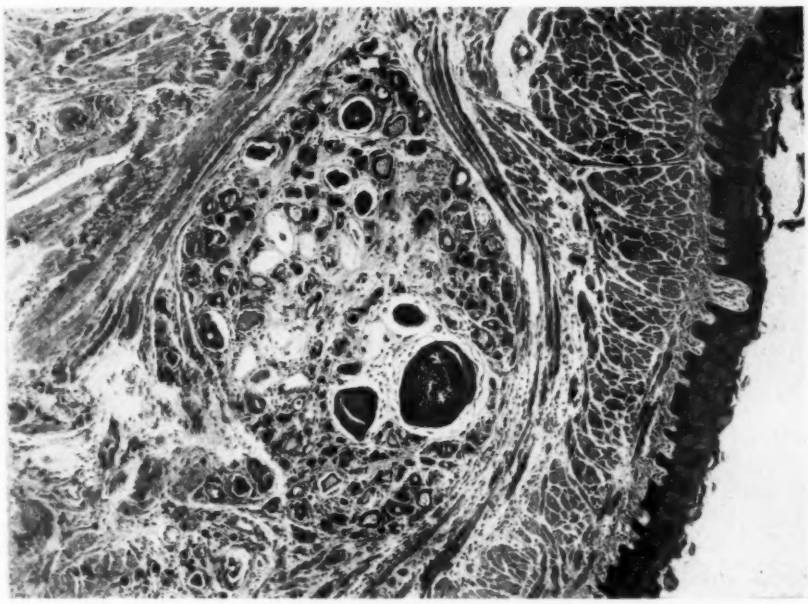

FIGURE 12.—Focus of calcified areas in tongue. × 63.

lesions of the tongue. Grossly, the lingual lesions occurred as one to five small round, discrete or confluent, white or gray, firm nodules 1 to 2 mm. in diameter, on the mucous surface of either or both sides of the tongue, usually on the dorsolateral surface. Grossly, the lesion was suggestive of papilloma; but microscopically, it consisted of a focus of hyalinized connective tissue embedded in which were numerous rounded deposits of calcium (fig. 12). These deposits were somewhat shrunken away from the periphery of the space in which they were embedded, which in turn was surrounded by a band of hyalinized connective tissue within which there were occasionally a few mononuclear cells. In some of the lesions there was a suggestion of calcification of deeply placed masses of squamous epithelium. The mucosa of the tongue overlying the calcific deposits was usually thickened and hyperkeratotic and occasionally showed papilloma formation, more especially when the calcific deposits were superficial. This calcific lesion of the tongue has been observed in untreated control mice of other strains kept under ordinary laboratory conditions. Whether the ingestion of methylcholanthrene was responsible for the marked frequency of this lesion in the experimental mice is still under consideration.

Induced pulmonary tumors were observed in both strains of mice. In the strain C3H mice, the number of tumors usually did not exceed 3 to 12 per mouse, whereas in many strain C mice 30 to 80 tumors were counted on the surface of each pair of lungs.

Papillomas and a few squamous-cell carcinomas about the mouth and face were observed in several mice. These tumors were probably associated with the direct contact between the skin and the

methylcholanthrene in the diet during periods of feeding.

There was usually marked atrophy of the genital and the lymphoid tissues in the experimental animals.

Of the miscellaneous lesions which occurred in some of the mice, the following might be mentioned: dehydration, pneumonia, pulmonary congestion, perirectal abscess, calculus in urinary bladder, necrosis of liver, and hyperplasia of esophageal mucosa. One strain C mouse had a cortical adenoma of the adrenal.

DISCUSSION

These data demonstrate that papillomas and squamous-cell carcinomas of the forestomach and precancerous lesions and adenocarcinomas of the small intestine can be induced in strains C and C3H mice by incorporating methylcholanthrene into the diet. The lesions are similar to those reported previously by Lorenz and Stewart (*1–3*) in other strains of mice in which the carcinogen was administered in the drinking water as an emulsion. A noteworthy observation based on the present material reveals that metastasis of the intestinal adenocarcinomas occurred not only to the mesentery and mesenteric lymph nodes but also to the liver and lung. The results tend to substantiate further the idea previously suggested by Lorenz and Stewart that these forestomach and intestinal tumors are the result of the direct action of methylcholanthrene on the tissue involved. The glandular mucosa of the stomach was totally refractory to tumor induction, and no tumors of the large intestine were obtained. Again these findings corroborate the data reported by the above-mentioned investigators.

Attesting to the hypothesis that methylcholanthrene is absorbed from the small intestine into the system is the occurrence of primary hemangio-endothelioma of the mesentery or mesenteric structures as well as the induction of primary pulmonary tumors. Of the two strains in the present experiment there was a much higher incidence of hemangio-endothelioma in the C mice than in the C3H. These tumors developed from tissues located along the pathway which the methylcholanthrene traversed following absorption from the small intestine. Tumors of blood-vessel origin have been found in mice of other strains ingesting the aqueous oil emulsions of either methylcholanthrene or dibenzanthracene. However, the two strains of mice used in the present experiment have not been sufficiently tested by the feeding of emulsions to allow comparisons to be made regarding the effectiveness of the different methods of administration. Andervont, Grady, and Edwards (*5*) have already reported the production of hemangio-endotheliomas readily in mice by the subcutaneous implantation of *o*-aminoazotoluene and have reviewed the subject of induced hemangio-endothelioma in the mouse. The oral administration of methylcholanthrene thus affords another convenient method of producing this type of lesion in strains C and C3H mice.

The papillomas and squamous-cell carcinomas about the face and neck that were observed in some of the experimental mice are probably attributable to the direct contact of the methylcholanthrene in the diet with the parts affected. The occurrence of skin tumors in this location is considerably less frequent when the carcinogen is administered in aqueous oil emulsions. This difference is probably due to the fact that mice receiving the aqueous oil emulsions have little occasion for spilling, whereas there is usually a certain degree of spillage of food which contains the methylcholanthrene.

SUMMARY

Fifty C3H and thirty-two C mice were fed methylcholanthrene (55 mg. per 100 gm. of diet) incorporated in the diet for 6 months after which time they were placed on the basal diet without methylcholanthrene. An average of 1 mg. of methylcholanthrene per day per mouse was ingested, and the mice were autopsied from 42 to 289 days after the start of the experiment.

Precancerous epithelial lesions and adenocarcinoma occurred in the small intestine; the intestinal carcinomas metastasized to the mesentery, mesenteric lymph nodes, pancreas, liver, and lung. Hemangio-endotheliomas involving singly or in combination the intestine, mesentery, pancreas, or pancreatic lymph nodes developed and metastasized to the liver and the lung.

Pulmonary tumors were induced in both strains of mice, with the higher incidence in the strain C mice.

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